

**Implications for coevolutionary dynamics of a tri-trophic
interaction between the orange-tip butterfly, its host plants
and primary parasitoid in a heterogeneous landscape**

**Thesis submitted in accordance with the requirements of the
University of Liverpool for the degree of Doctor in Philosophy by
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Preface

This thesis grew out of field work undertaken by myself in my local nature reserve from the year 2003 onwards. Initially, my aim was to investigate the frequency of occurrence of dwarf orange-tip butterflies, which had been unusually abundant the year before. Over the course of the next five years my interests widened and my data-set expanded, such that by 2008 I felt that I had publication-worthy material. To this end I got in touch with Dr. Ilik Saccheri at the University of Liverpool. It rapidly became clear to us that the best way forward would be for me to enrol as a doctoral candidate under Dr Saccheri's supervision. I mention this pre-history to emphasize that this work differs in origin from a typical doctoral thesis and that since it was not funded by a research council many interesting questions which could have been pursued in relation to it remained economically out-of-bounds.

Over the past seven years Dr. Saccheri has patiently guided me through two publications and the preparation of this manuscript. His constructive comments on the (numerous) drafts of these works have been very helpful in shaping my ideas. Our two published papers form the basis for Chapters 2 and 3 of this thesis; all observations and experiments incorporated from them are my own, as well as the basic form of the text. I am also indebted to Prof. David Thompson, Prof. Greg Hurst, Prof. Geoff Parker and Dr. Jenny Hodgson for reading and providing constructive criticisms on earlier drafts for parts of this work, and to Dr. Stephen Cornell for checking (and correcting) some of the mathematics here presented. Prof. Steve Paterson and Dr. Phill Watts provided useful feedback on my 1st and 2nd year reports (actually submitted in the 2nd and 4th years of a part time course). Prof. Mike Singer and several anonymous reviewers have been responsible for improving material submitted for publication. Dr. Hugh McAllister and Tim Baxter have assisted my work at Ness Gardens. I am very grateful to the Rangers at Dibbinsdale Nature Reserve, Peter Miller and Alan Smail, for permission to undertake my field-work there, and for the interest they took in it.

My parents, Tom and Carol, have always been very supportive and encouraging of my scientific endeavours. To them I owe a huge debt. My father will doubtless take his usual interest in my thesis. Sadly, my mother did not live to see it completed; I dedicate the work to her memory. I thank my sister, Lucy, and brother-in-law, Mike,

for the interest they have taken in my work. To other members of my family, now deceased, I am indebted for much generosity and kindness over the years: to my great aunt and great uncle, Nancie and Fred, and to my grandparents, Nellie, Ronnie and Tom.

Contents

Title page.....	i
Preface.....	ii
Contents.....	iv
Dedication.....	viii
Abstract.....	ix
Chapter 1. Introduction.....	1
Life-history of organisms comprising study system.....	4
Autotrophs.....	4
Herbivore.....	6
Parasitoid.....	7
Study location.....	8
Ecological characteristics of sub-sites.....	9
1. Spital.....	9
2. Otters' Tunnel.....	10
3. Lower Tip.....	11
4. Upper Tip.....	11
5. Boden's Hey.....	12
6. Lady Bridge.....	13
7. Marford's Wood.....	14
Butterfly species recorded in Dibbinsdale.....	15
The geographical selection mosaic: what we know.....	15
Chapter 2. Effect of <i>Anthocharis cardamines</i> host-plant utilization on body-size, eclosion timing and dispersal.....	24
Abstract.....	24
Introduction.....	24
Methods.....	26
Effect of host-plant on body-size.....	26
Timing of eclosion.....	27
Dispersal-rate.....	27
Statistical analyses.....	29
Results.....	30
Host-plant use and size variation.....	30
Eclosion time.....	31
Size-dependent dispersal.....	35
Discussion.....	39
Chapter 3. Male emergence schedule and dispersal behaviour are modified by mate availability in heterogeneous landscapes.....	44
Abstract.....	44

Introduction	44
Methods	50
POPAN estimation of male and female emergence schedules.....	50
Male fitness model.....	51
Reanalysis of data from Parker and Courtney (1983).....	54
Intra-seasonal changes in male behaviour.....	54
Theory.....	54
Method.....	56
Results	57
Discussion	63
Interpretation of results.....	63
Evolution of emergence timing and dispersal in heterogeneous landscapes.....	65
Implications for conservation: maintenance of sink populations.....	68
The 'stay-or-go' response and geographical selection mosaics.....	69
 Chapter 4. Impact of parasitism by <i>Phryxe vulgaris</i> on life-history evolution in <i>Anthocharis cardamines</i>	71
Abstract	71
Introduction	71
Methods	75
Theoretical considerations.....	75
Size-fecundity relation.....	77
Larval growth parameters.....	77
Mathematical models.....	77
Results	83
Size-fecundity relation.....	83
Larval growth-curves.....	84
Relative duration of exponential growth phase.....	85
Predictors of 4th and 5th larval instar period.....	87
Mathematical modelling parameters.....	89
Fitness vs. wing-length.....	91
Combined effect of size and resting phase duration.....	92
Effect of host use.....	96
Discussion	98
Impact of parasitism on body-size evolution.....	98
Evolution of larval resting phase behaviour.....	101
Impact of host use.....	102
Conclusions	103
 Chapter 5. Evolution of flowering phenology in <i>Cardamine pratensis</i> in response to pre-dispersal seed predation by <i>Anthocharis cardamines</i>	104
Abstract	104
Introduction	104
Methods	108

Host-plant transects.....	108
Survival of <i>A. cardamines</i> immature stages.....	109
Results.....	109
Discussion.....	119
 Chapter 6. Evolution of adaptive phenotypic plasticity and advancement in phenology of <i>Anthocharis cardamines</i> with respect to its host-plant <i>Cardamine pratensis</i>.....	 125
Abstract.....	125
Introduction.....	125
Methods.....	129
Results.....	131
Longitudinal changes in phenology and body-size.....	131
Body-size phenotypic plasticity on <i>C. pratensis</i>	132
Longitudinal depression of wing-length on localized spatial and temporal scales.....	136
Phenological interaction of male and female <i>A. cardamines</i> and <i>C. pratensis</i>	139
Heritability of <i>A. cardamines</i> wing-length and eclosion date.....	143
Discussion.....	144
 Chapter 7. Discussion.....	 149
 Appendices.....	 160
1. To show that the resettlement-rates imply that small specimens were less dispersive than larger ones in the WS in 2006 and 2007.....	160
2. Evolution of emergence timing in isolated populations in response to depressed dispersal-rate.....	161
3. Critique of Courtney's (1982b) hypothesis on the maintenance of polyphagy in <i>A. cardamines</i>	163
4. Derivation of predicted fraction of recaptures, F	165
5. Independence of 'stay-or-go' response from body-size (wing-length).....	169
6. Variance in source emergence timing and maintenance of sink populations.....	170
7. Threshold larval size for parasitoid attack and variable infection rates in Cheshire.....	176
8. Derivation of equations (4.9) and (4.12) in Chapter 4.....	176
(i) Derivation of equation (4.9) for $\ln(S_T)$ when parasitism is restricted to the fifth instar.....	177
(ii) Derivation of equation (4.12) for $\ln(S_T)$ when parasitism extends over the fourth and fifth instars.....	177
9. To determine whether the disappearance of final instar larvae before the resting phase is due to early vacation of the host-plant or predation.....	178
10. Evolution of <i>C. pratensis</i> flowering time in response to selection by egg-laying <i>A. cardamines</i> females: a model.....	180

References.....186

To the Memory of my Mother
Carol Davies

Implications for coevolutionary dynamics of a tri-trophic interaction between the orange-tip butterfly, its host plants and primary parasitoid in a heterogeneous landscape

William James Davies

Abstract

Coevolutionary interactions change across landscapes, leading to the formation of geographic selection mosaics. Analyses of coevolutionary dynamics have so far focused on interactions between two trophic levels. The tritrophic interactions between the pierid butterfly *Anthocharis cardamines* (the orange-tip), its brassicaceous host-plants *Cardamine pratensis* (lady's smock) and *Alliaria petiolata* (garlic mustard), and primary parasitoids *Phryxe vulgaris* (Tachinidae) and *Cotesia saltator* (Braconidae), are known to vary across mainland Britain and Continental Europe. In Britain, northern *A. cardamines* populations tend to utilize *C. pratensis* and southern ones *A. petiolata*; the primary parasitoid in this country is *P. vulgaris*. In Sweden, the butterfly does not show a strong preference for any brassicaceous host-plant, and the primary parasitoid is *C. saltator*. In this thesis, I investigate likely selection pressures operating on these interactions in a single study site on the Wirral peninsula in northern England. Host use affects the emergence timing and dispersal of *A. cardamines* males; specimens utilizing *C. pratensis* emerge earlier than those utilizing *A. petiolata*, and are also smaller. Since small size is linked with depressed dispersal, utilization of *C. pratensis* results in an "emerge early and wait" mate seeking strategy; conversely, utilization of *A. petiolata* is associated with an "emerge late and rove" strategy. These alternative strategies are likely adapted to the varying density of locally emerging females across the landscape, with high density populations tending to be associated with *C. pratensis* and low density ones with *A. petiolata*. In dense populations, late emerging males will be at a disadvantage since females mate only once; in the study population, their predicted fitness always declines to <1 in late season. This is coupled with a 'stay-or-go' response, in which a proportion of late emerging males immediately emigrate to a low density continuum outside the study area, where early emergence is less critical for fitness. Such a response could help maintain sink populations by averting Allee effects (decreased population growth due to low mate encounter rates). Late instar *A. cardamines* larvae are heavily parasitized by *P. vulgaris*. This could select for early vacating of the host-plant at small larval size, whereas the size-fecundity relationship in females should select for prolonged growth to larger size. Mathematical models indicate that high rates of parasitization are sufficient to overturn the fecundity benefit of large size, but fall short of maintaining strong stabilizing selection for an optimal wing-length. The tendency of some larvae to move off their host-plant before the final instar resting phase is probably a direct evolutionary response to parasitism risk. *A. cardamines* larvae are pre-dispersal seed-predators; an early flowering ecotype of *C. pratensis* has likely been selected to avoid egg-laying *A. cardamines* females. In turn, the butterfly appears to be invading this host-plant's phenological space, with selection favouring small, early emerging females which oviposit on it. This cautions against interpreting the recent advance in *A. cardamines*' phenology solely in terms of a response to climate change. Spatio-temporal variation in the intensity of these effects likely contributes to the ongoing coevolutionary dynamics within this tritrophic system.

Chapter 1

Introduction

The role of evolutionary processes in shaping ecosystem structure and function is poorly understood (Thompson, 2009). The structure and dynamics of food webs within ecosystems depends partly on coevolutionary interactions between the organisms composing them (Thompson 2005, 2009). The geographic mosaic theory of coevolution (Thompson, 2005) posits that these interactions change across ecosystems; alterations in the distribution and abundance of species, as well as changes in the environmental and community contexts in which they interact, alter the structure of selection among communities and result in the formation of geographic selection mosaics. Hence, the interaction between a pair of organisms is fundamentally a genotype x genotype x environment interaction. In addition, variation in the strength of reciprocal selection generates geographical hotspots and coldspots in the coevolutionary interaction between species. The structure of food webs is therefore dynamic and inconstant among ecosystems. The continual reshaping of interactions across landscapes is aided by gene flow, genetic drift and metapopulation dynamics, which redistribute traits among populations (Thompson, 2005).

Geographic (phenotypic) selection mosaics have been demonstrated in both insect-plant and parasite-host interactions. Thus, in western North America, the interaction between the moth *Greya politella* (Prodoxidae) and the herbaceous plant *Lithophragma parviflorum* (Saxifragaceae) ranged from mutualistic to antagonistic among 12 sites in Idaho, Oregon and Washington (Thompson and Cunningham, 2002). The moths interact with the plants as both pollinators and floral parasites (larvae consume a small proportion of seeds); in localities where *L. parviflorum* co-pollinators are rare, its relationship with *G. politella* is mutualistic, and in localities where they are common, the relationship is either commensal or antagonistic. Similarly, the structure of selection governing the coevolutionary interaction between *Polistes* wasps (*P. biglumis*) and their congeneric social parasites (*P. atrimandibularis*) varies among populations in the Alps and Apennines due to differences imposed by predators (Lorenzi and Thompson, 2011).

These results demonstrate that pairwise coevolutionary interactions can be dynamic and that the data obtained from field studies must be interpreted within a localized context. However, food webs consist of an assemblage of pairwise interactions, and the role of coevolution in shaping multitrophic interactions has been neglected, primarily because selection is considered too diffuse to shape multiple interactions simultaneously. This view is at variance with the fact that organisms can be simultaneously adapted to multiple facets of the abiotic environment (temperature, humidity, photoperiod, etc.), and there is no reason to suppose that similarly wide ranging adaptations to selection pressures imposed by multiple biotic agents cannot be combined, or at least trade-off, in the same organism (Thompson, 2005).

The food web consisting of the two Brassicaceous herbs *Cardamine pratensis* L. and *Alliaria petiolata* Bieb. (autotrophs), the Pierid butterfly *Anthocharis cardamines* L. (herbivore), and the Tachinid and Braconid parasitoids *Phryxe vulgaris* Fallen and *Cotesia saltator* (Thunberg) comprises a good system to study pairwise interactions within a tritrophic context. In Britain, *C. pratensis* (lady's smock or cuckooflower) and *A. petiolata* (garlic mustard or jack-by-the-hedge) are the principal host-plants of *A. cardamines* (the orange-tip), although it occasionally utilizes a wide range of alternative Brassicaceous (and sometimes Resedaceous) herbs (Courtney and Duggan, 1983). There is an apparent change in host preference across the British mainland; southern and eastern *A. cardamines* populations tend to utilize *A. petiolata* and northern and western ones *C. pratensis* (Courtney and Duggan, 1983). Moreover, in Sweden, the butterfly is more truly polyphagous, and does not exhibit a strong preference for any particular Brassicaceous host (Wiklund and Åhrberg, 1978). Late instar *A. cardamines* larvae are heavily attacked by *P. vulgaris* in some British localities, but not in others (Courtney and Duggan, 1983, Dempster, 1997); in Sweden, *P. vulgaris* has not been recorded to infect *A. cardamines*, where its place is taken by *C. saltator* (Wiklund and Åhrberg, 1978, Courtney, 1980; Wiklund and Friberg, 2009). The change in *A. cardamines*' host use across the British mainland, and in its degree of polyphagy and the parasitoid species attacking it across Europe, hint at the simultaneous occurrence of geographic selection mosaics with respect to the plant-herbivore and herbivore-parasitoid interactions within this tritrophic system.

Accurate interpretation of the geographical variation in the structure and dynamics of these interactions requires the establishment of the likely selection pressures shaping their coevolution. In Britain, it is known that *A. cardamines* pupal mass is affected by host use (Courtney, 1981), and that this in turn impacts the fecundity of females (Courtney 1981, Duggan, 1985). The size variance exhibited by this species is unusual (Fig. 1.1) and dwarf specimens are of widespread occurrence (Williams, 1915; Wiklund and Ahrberg, 1978); dwarf males have been occasionally observed to emerge earlier than normal sized ones in the field (Newman, 1869; Barrett, 1888; Ford, 1945). In this study, I shall build on these observations to try to identify the selection pressures likely to be operating on the plant-herbivore and herbivore-parasitoid interactions in this system. For a single study site in northern England, I shall attempt to answer four key questions:

1. Does the size variance resulting from utilizing different host-plant species impact the emergence timing and dispersal of *A. cardamines*?
2. How is the fitness of *A. cardamines* males affected by their emergence timing?
3. Has the growth and behaviour of *A. cardamines* larvae on the host-plants evolved in response to the risk of parasitism by *P. vulgaris*?
4. Have the flowering times of the host-plants evolved in response to the risk of exposure to egg-laying *A. cardamines* females?

Geographical variation in the strength of the plant-herbivore and herbivore-parasitoid interactions will likely affect the fine-scale evolutionary adjustment of some or all of these traits. This could impact conservation issues. Since the phenologies of the orange-tip and lady's smock/garlic mustard can be used to track global warming (Sparks and Yates, 1997; Phillimore et al., 2012; Diamond et al., 2011; Brooks et al., 2014; Navarro-Cano et al., 2015), the coevolution of the butterfly's emergence timing with the plants' flowering phenology could complicate the interpretation of phenological data. Moreover, dispersal ability is critical for the persistence of species and the maintenance of their genetic diversity in fragmented landscapes (e.g. Vandewoestijne et al., 2004; Ronce, 2007; Gibbs et al., 2010). Hence, the dynamic evolution of geographic selection mosaics is likely an important (and currently

overlooked) component in species' responses to habitat fragmentation and climate change. I shall therefore attempt to answer a fifth question in relation to these issues:

5. Is there evidence that coevolutionary interactions which affect emergence timing and dispersal could impact *A. cardamines*' persistence in fragmented landscapes and phenological response to climate change?



Figure 1.1 Size variation in male *A. cardamines*. Both specimens were captured in Dibbinsdale Nature Reserve in 2003. The upper specimen has a forewing length of 17 mm, the lower one 21 mm. In the Dibbinsdale population, male wing-length varies between 15 mm and 22 mm (female wing-length between 15 mm and 24 mm).

Life-history of organisms comprising study system

Autotrophs

In addition to *A. petiolata* and *C. pratensis*, I append a brief description of *Barbarea vulgaris* R. Br. (wintercress or yellow rocket), a third Brassicaceous host occasionally utilized by *A. cardamines* in my study population.

Alliaria petiolata. (Fig. 1.2, left) A biennial herb, distributed throughout the British Isles except the Highlands and Islands of Scotland (Blamey, Fitter and Fitter, 2003), and probably native to it (first recorded in Roman times) (Clapham, Tutin and Moore, 1989). Flowers April-June. Grows in a wide variety of habitats on nutrient rich soils, preferring light shade; especially common in woods and along hedgerows and riverbanks.

Cardamine pratensis. (Fig. 1.2, *centre*) A perennial herb distributed throughout the British Isles (Blamey, Fitter and Fitter, 2003), where it is native (Clapham, Tutin and Moore, 1989). Flowers March-July. Grows in moist habitats up to 1000 m in Scotland; common in woods, pastures, damp meadows and reed-beds. An aggregate species forming a taxonomically difficult polyploid complex; octaploids are most common in Britain (Dale and Elkington, 1974).

In my study site (Dibbinsdale Nature Reserve) there are two ecotypes of *C. pratensis*, a small early flowering form and a large late flowering one; for convenience, these will be termed "small" *C. pratensis* and "large" *C. pratensis* respectively. The small ecotype occurs commonly throughout the Reserve but the large one is restricted to a single sub-site (the "Upper Tip" - see below) from which small *C. pratensis* is absent. These ecotypes will be described in more detail in Chapter 5.

Barbarea vulgaris (Fig. 1.2, *right*). A biennial or perennial herb distributed throughout the British Isles except the Highlands and Islands of Scotland and the mountainous regions of Wales (Blamey, Fitter and Fitter, 2003), where it is native (Clapham, Tutin and Moore, 1989). Flowers May-August. Grows in damp places along hedges and streamsides.



Figure 1.2 *Alliaria petiolata* (left) in Dibbinsdale Nature Reserve (Otters' Tunnel sub-site) on 30 April 2012 (James Davies); *Cardamine pratensis* (centre) in Dibbinsdale (Spital sub-site) on 7 May 2013 (James Davies); *Barbarea vulgaris* (right) in Kerava, Finland on 20 May 2009 (Anneli Salo, https://commons.wikimedia.org/wiki/File:Barbarea_vulgaris_Peltokanankaali_IM8338_C.JPG)

Herbivore

Anthocharis cardamines (Fig. 1.3) is a univoltine insect distributed throughout the British Isles, but only patchily (and absent from the Isles) in Scotland (UK Butterflies, <http://www.ukbutterflies.co.uk>). Its range contracted southwards from the late 19th century onwards (Long, 1979) and by the mid 20th century it had disappeared from most northern localities except for an isolated stronghold in Aberdeenshire (Courtney, 1980; Asher et al., 2001); its subsequent re-expansion in the late 20th century (Long, 1979) is likely ongoing. It is a butterfly of open woods, damp fields, lanes and riverbanks; the flight period extends six to seven weeks from April to June. Males adopt patrolling behaviour to find females; in my study population they gradually disperse by increasing the range or shifting the location of their patrolling ground. Larvae (Fig. 1.3, *right*) feed on the host-plants in May and June, which are vacated at the end of the fifth (final) instar, pupation taking place in concealed locations. Aestivation/hibernation is passed in the pupal stage.

The species is strongly sexually dimorphic (Fig. 1.3, male *left*, female *centre*), and distinct subspecies have been described from Ireland (ssp. *hibernica* Williams) and the British mainland (ssp. *britannica* Verity). The phenotypic differences between ssp. *britannica* and the races occurring in Continental Europe and the Middle East are very slight, but they differ in chromosome number (the haploid count in *britannica* is $n = 30$ instead of $n = 31$); a fusion of two chromosomes in *britannica* since the separation of southern England from the Continent 7000 years ago appears to be the most likely explanation (Bigger, 1978).



Figure 1.3 Adult male (left), female (centre) and larva (right) of *Anthocharis cardamines*. The male is a dwarf (wing-length = 15 mm) photographed in Dibbinsdale Nature Reserve (Spital sub-site) on 12 May 2006; the female (wing-length = 21 mm) was photographed in Dibbinsdale (Spital sub-site) on 13 May 2012; the larva was photographed in Dibbinsdale (Upper Tip sub-site) on 12 June 2012 (all photographs by James Davies).

Parasitoid

To *P. vulgaris*, I append a description of *Phryxe nemea*, which can easily be mistaken for it and which also attacks *A. cardamines*' larvae; records in the literature are therefore likely to be confused. All specimens reared in my experiments were confirmed as *P. vulgaris*.

Phryxe vulgaris (Fig. 1.4). A multivoltine polyphagous parasitoid of larger Lepidopterous larvae, with about half the records from Nymphalidae and Pieridae (Belshaw, 1993). It attacks exposed larvae feeding on herbs; small, hairy or arboreal species avoided (Ford and Shaw, 1991). The adult flies visit flowers in open habitats throughout the British Isles from May to September, peaking in August (Belshaw, 1993). Eggs are attached to the skin of the host, hatching rapidly. Larvae immediately bore into the host to feed on the blood and fat-body, and later on the vital organs (Imms, 1947), when the host is killed. Mature larvae leave the host to pupate in the earth. Pupae hibernate, or third instar larvae may overwinter within host (Belshaw, 1993).



Figure 1.4 *Phryxe vulgaris* in Cholsey, Oxfordshire. Photograph by Chris Raper (Tachinid Recording Scheme, <http://tachinidae.myspecies.info/taxonomy/term/152>).

(*Phryxe nemea* Meigen is also a multivoltine polyphagous parasitoid of Lepidopterous larvae, closely related to *P. vulgaris*. Distributed throughout the British Isles in deciduous woodland and scrub (Belshaw, 1993). Flies from early May to early October, peaking in May (Belshaw, 1993). Rarely attacks microlepidoptera; frequently reared from *Abraxas grossulariata* (magpie moth) in Britain (Ford and Shaw, 1991). A few British records from *A. cardamines*.)

Study location

Dibbinsdale Nature Reserve is located in the Wirral peninsula, Cheshire, England (53.3°N, 3.0°W; Fig. 1.5). It encompasses a considerable remnant (475 ha) of semi-natural ancient woodland situated in an incised valley formed by Dibbinsdale Brook cutting through a layer of boulder clay left from the last ice age into sandstone bedrock. The woodland alternates with reed-beds and other open areas which are often inundated after heavy rain. The damp habitat is well suited to *C. pratensis*, which grows in large patches throughout the Reserve; *A. petiolata* is more thinly spread through the Reserve, but is still abundant. The locality is long and thin in profile, and follows the course of Dibbinsdale Brook for about 1.5 km as it flows northwards to the River Mersey. It is semi-isolated with respect to the ecological requirements of *A. cardamines*, being flanked by farmland and urban areas (Fig. 1.5).

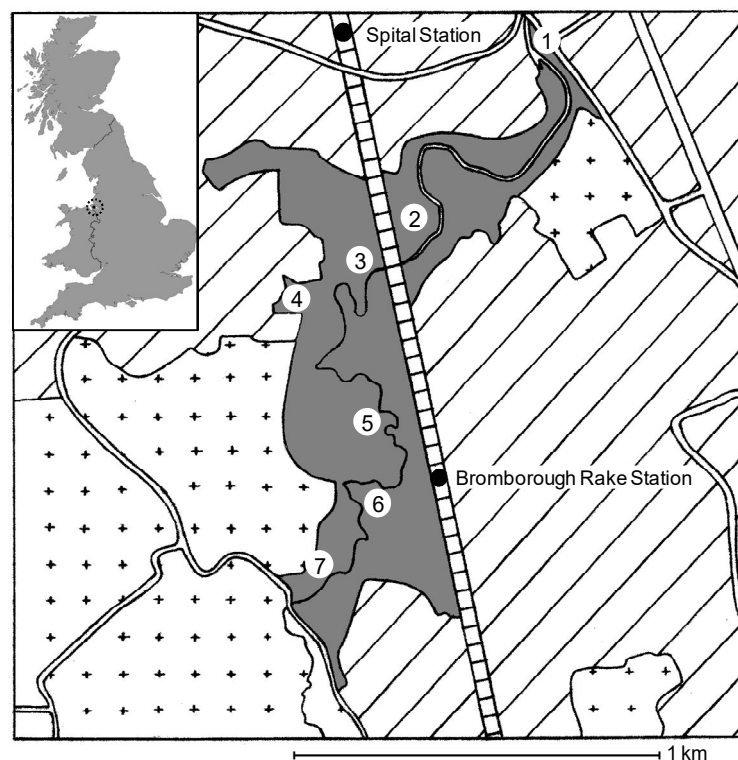


Figure 1.5 Dibbinsdale Nature Reserve (in grey, with Dibbinsdale Brook running through it) and surrounding area, showing the main sampling locations (sub-sites 1-7) for *A. cardamines*. Hatched lines indicate urbanized areas, crosses open fields and agricultural land, and the ladder a railway track. The approximate location of the Reserve is indicated on the inset map of the British mainland.

Sampling of adult *A. cardamines* was largely restricted to seven sub-sites within the Reserve (Fig. 1.5), containing large patches of *C. pratensis* or *A. petiolata*. As well as being the principal hosts, these plants are often utilized as nectar sources by the

imagines; the patches were regularly visited by the males. Each patch was separated from its nearest neighbour by distance or, in one case, a railway track (Fig. 1.5). The distances between all sub-sites, obtained from the Global Positioning System, are given in Table 1.1.

Table 1.1 Inter-sub-site distances in Dibbinsdale Nature Reserve.

	1 S	2 OT	3 LT	4 UT	5 BH	6 LB	7 MW
1 S	-	560 m	752 m	908 m	1.1 km	1.3 km	1.5 km
2 OT	560 m	-	195 m	361 m	566 m	745 m	995 m
3 LT	752 m	195 m	-	171 m	415 m	582 m	837 m
4 UT	908 m	361 m	171 m	-	390 m	525 m	776 m
5 BH	1.1 km	566 m	415 m	390 m	-	240 m	454 m
6 LB	1.3 km	745 m	582 m	525 m	240 m	-	255 m
7 MW	1.5 km	995 m	837 m	776 m	454 m	255 m	-

Letters are initials of designated place names: S - Spital; OT - Otters' Tunnel; LT - Lower Tip; UT - Upper Tip; BH - Boden's Hey; LB - Lady Bridge; MW - Marford's Wood.

Ecological characteristics of sub-sites

Each sub-site is designated by both a number (Fig. 1.5) and a place name (Table 1.1).

1 Spital.

BNG coordinates: SJ 344 831.

Ecological characteristics: A woodland path (Fig. 1.6) bordered on one side by a dense covering of *Rubus fruticosus* and *Urtica dioica* (invaded by trees) with *Cardamine pratensis* under the bramble, and on the other side by more bramble and nettle under a line of trees; *Alliaria petiolata* in dense clumps either side of the woodland path.

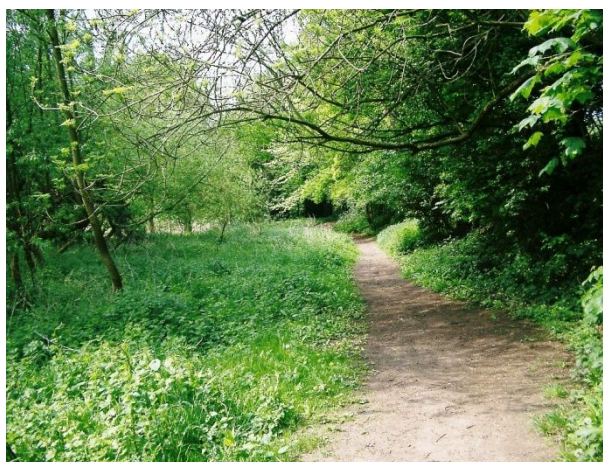


Figure 1.6 Woodland path at Spital (photographed on 12 May 2006 by James Davies).

Spring plants recorded at sub-site: *Ranunculus ficaria*, *Urtica dioica*, *Rumex*, *Alliaria petiolata*, *Cardamine pratensis*, *Rubus fruticosus*, *Heracleum sphondylium*, *Anthriscus sylvestris*, *Galium aparine*, *Taraxacum officinale*, *Hyacinthoides non-scriptus*, *Salix*, *Ulmus glabra*, *Fraxinus excelsior*, *Sambucus nigra*, *Quercus*, *Crataegus monogyna*, *Acer pseudoplatanus*, *Hedera helix*, *Ilex aquifolium*.

2 Otters' Tunnel.

BNG coordinates: SJ 341 826.

Ecological characteristics: A dense stand of *Phragmites australis* (which, in early spring, consists of the dried remains of the previous years' growth) bordered and invaded by carr woodland, and cut through by a raised path with *Cardamine pratensis*, *Alliaria petiolata*, *Urtica dioica*, *Taraxacum officinale* and *Anthriscus sylvestris* along its borders (Fig. 1.7). Patches of *C. pratensis* also in small enclaves within the reed-bed; *Barbarea vulgaris* rare.



Figure 1.7 Raised path cutting through the reed-bed at Otters' Tunnel; *C. pratensis* plants can be seen growing in the green verge at bottom left (photographed on 27 April 2006 by James Davies).

Spring plants recorded at sub-site: *Ranunculus repens*, *R. ficaria*, *Urtica dioica*, *Silene dioica*, *Rumex*, *Barbarea vulgaris*, *Alliaria petiolata*, *Cardamine pratensis*, *C. flexuosa*, *Chrysosplenium oppositifolium*, *Geum urbanum*, *Mercurialis perennis*, *Anthriscus sylvestris*, *Veronica chamaedrys*, *V. montana*, *Plantago lanceolata*, *Galium aparine*, *Taraxacum officinale*, *Alnus glutinosa*, *Populus*, *Salix*, *Fraxinus excelsior*, *Quercus*, *Crataegus monogyna*, *Acer pseudoplatanus*, *Hedera helix*,

Phragmites australis, *Equisetum sylvaticum*; also early growth of *Filipendula ulmaria* and *Impatiens glandulifera*.

3 Lower Tip.

BNG coordinates: SJ 339 829.

Ecological characteristics A wet and muddy woodland clearing with a carpeting of *Cardamine pratensis* and *Urtica dioica* amongst reed-bed on a firmer slope of raised ground (Fig. 1.8); also clumps of *Alliaria petiolata* in more shaded areas.



Figure 1.8 The slope of raised ground (right) at the Lower Tip (photographed by James Davies on 28 April 2006). *Cardamine pratensis* is present but difficult to distinguish in the photograph.

Spring plants recorded at sub-site: *Ranunculus ficaria*, *Anemone nemorosa*, *Urtica dioica*, *Rumex*, *Alliaria petiolata*, *Cardamine pratensis*, *C. flexuosa*, *Rubus fruticosus*, *Mercurialis perennis*, *Geranium robertianum*, *Heracleum sphondylium*, *Anthriscus sylvestris*, *Galium aparine*, *Taraxacum officinale*, *Hyacinthoides non-scriptus*, *Corylus avellana*, *Betula pendula*, *Populus alba*, *Salix*, *Quercus*, *Crataegus monogyna*, *Acer pseudoplatanus*, *Hedera helix*, *Phragmites australis*, *Phyllitis scolopendrium*.

4 Upper Tip.

BNG coordinates: SJ 339 825.

Ecological characteristics: A small area of damp meadowland (the dried remains of the previous years' growth of *Dactylis glomerata* being especially prominent in the

spring-time) bordered by *Rubus fruticosus* and trees. The large ecotype of *Cardamine pratensis* is restricted to this sub-site, where it occurs abundantly with *Taraxacum officinale* in grassy areas (Fig. 1.9); these are joined later by *Ranunculus acris* and *R. repens*.

Spring plants recorded at sub-site: *Ranunculus acris*, *R. repens*, *Cerastium fontanum*, *Rumex obtusifolius*, *Rumex acetosa*, *Cardamine pratensis*, *Rubus fruticosus*, *Trifolium pratense*, *Heracleum sphondylium*, *Veronica chamaedrys*, *V. filiformis*, *Plantago lanceolata*, *Taraxacum officinale*, *Fraxinus excelsior*, *Quercus*, *Acer pseudoplatanus*, *Phleum bertolonii*, *Dactylis glomerata*.



Figure 1.9 Abundant dandelions (*Taraxacum officinale*) at the Upper Tip (photographed by James Davies on 6 May 2006). The large ecotype of *Cardamine pratensis* is also present but difficult to distinguish in the photograph.

5 Boden's Hey.

BNG coordinates: SJ 341 819.

Ecological characteristics: A long stretch of riverbank habitat by Dibbinsdale Brook with dense patches of *Cardamine pratensis*, *Alliaria petiolata* and (less commonly) *Barbarea vulgaris* interspersed among trees, nettles and reed-bed (Fig. 1.10). Bounded by open meadowland on one side and a steep densely wooded slope on the other. Orange-tips move freely among various sampling points within this sub-site; there is no point in sub-dividing it further.



Figure 1.10 Female *Anthocharis cardamines* at rest on *Alliaria petiolata* on the bank of Dibbinsdale Brook in Boden's Hey (photographed by James Davies on 30 April 2012).

Spring plants recorded at sub-site: *Ranunculus repens*, *R. ficaria*, *Anemone nemorosa*, *Urtica dioica*, *Silene holostea*, *Rumex*, *Barbarea vulgaris*, *Alliaria petiolata*, *Cardamine pratensis*, *Rubus fruticosus*, *Claytonia sibirica*, *Geranium robertianum*, *Heracleum sphondylium*, *Anthriscus sylvestris*, *Aegopodium podagraria*, *Conopodium majus*, *Symphytum officinale*, *Plantago lanceolata*, *Galium aparine*, *Cirsium arvense*, *Taraxacum officinale*, *Hyacinthoides non-scriptus*, *Corylus avellana*, *Alnus glutinosa*, *Salix*, *Crataegus monogyna*, *Acer pseudoplatanus*, *Phragmites australis*, *Phleum bertolonii*, *Dactylis glomerata*, *Pteridium aquilinum*; early growth of *Impatiens glandulifera*.

6 Lady Bridge.

BNG coordinates: SJ 339 819.

Ecological characteristics: Raised ground punctuated with ponds and bounded by the course of Dibbinsdale Brook. *Salix* trees on a small "island" in the central area; *Alliaria petiolata* abundant, *Cardamine pratensis* and *Barbarea vulgaris* present; early (and prolific) growth of *Impatiens glandulifera* and some *Anthriscus sylvestris* (Fig. 1.11).



Figure 1.11 A small enclave at Lady Bridge with *Anthriscus sylvestris* (photographed by James Davies on 24 May 2006).

Spring plants recorded at sub-site: *Ranunculus repens*, *Urtica dioica*, *Silene dioica*, *Rumex obtusifolius*, *Persicaria bistorta*, *Barbarea vulgaris*, *Alliaria petiolata*, *Cardamine pratensis*, *Geum urbanum*, *Claytonia sibirica*, *Anthriscus sylvestris*, *Galium aparine*, *Allium ursinum*, *Alnus glutinosa*, *Salix*, *Fraxinus excelsior*, early growth of *Impatiens glandulifera*.

7 Marford's Wood.

BNG coordinates: SJ 339 817.

Ecological characteristics: Three adjacent areas: a tree-lined riverbank habitat with dense stands of *Alliaria petiolata* (Fig. 1.12, *left*), a damp, muddy, open woodland (sometimes with a small lake) carpeted by *Urtica dioica* and *Cardamine pratensis* (Fig. 1.12, *right*), and an open clearing with *Pteridium aquilinum* and *Hyacinthoides non-scriptus*.



Figure 1.12 *Left*: Dense stand of *Alliaria petiolata* on riverbank (river out of sight at right) in Marford's Wood (photographed by James Davies on 14 May 2012). *Right*: Carpeting of *Urtica dioica* with *Cardamine pratensis* in open woodland (a drainage pipe runs diagonally across top of picture) in Marford's Wood (photographed by James Davies on 20 April 2007).

Spring plants recorded at sub-site: *Anemone nemorosa*, *Urtica dioica*, *Silene dioica*, *Alliaria petiolata*, *Cardamine pratensis*, *C. flexuosa*, *Rubus fruticosus*, *Oxalis acetosella*, *Heracleum sphondylium*, *Anthriscus sylvestris*, *Taraxacum officinale*, *Allium ursinum*, *Hyacinthoides non-scriptus*, *Corylus avellana*, *Alnus glutinosa*, *Salix*, *Acer pseudoplatanus*, *Pteridium aquilinum*.

Butterfly species recorded in Dibbinsdale

An asterisk or crucifix indicates that *P. vulgaris* or *P. nemea* (respectively) have been reared from the indicated species in Britain.

*Thymelicus sylvestris**, *Ochlodes venata*, *Gonepteryx rhamni*†, *Pieris brassicae**, *P. rapae**, *P. napi**, *Anthocharis cardamines**†, *Quercusia quercus*†, *Satyrrium w-album*†, *Lycaena phlaeas**, *Polyommatus icarus*, *Celastrina argiolus*, *Vanessa atalanta*†, *Cynthia cardui**, *Aglais urticae**†, *Inachis io**, *Polygonia c-album*, *Pararge aegeria*†, *Pyronia tithonus*, *Maniola jurtina*, *Aphantopus hyperanthus*.

The geographical selection mosaic: what we know

I here summarize what is known of the interactions between *A. cardamines*, its host-plants and parasitoids.

In Continental Europe *A. cardamines* is polyphagous (Wiklund and Åhrberg, 1978), but in the British mainland it is more specialized on the two host-plants *A. petiolata* and *C. pratensis* (Courtney and Duggan, 1983). Although the butterfly can utilize a wide range of Brassicaceous plants in Britain, in a survey conducted by Courtney

and Duggan (1983) nearly half the records from correspondents were for *A. petiolata* and *C. pratensis* (Fig. 1.13). This represents an underestimate of the frequency with which these hosts are utilized, since these data are unweighted for the number of eggs laid on each host species within the surveyed localities.

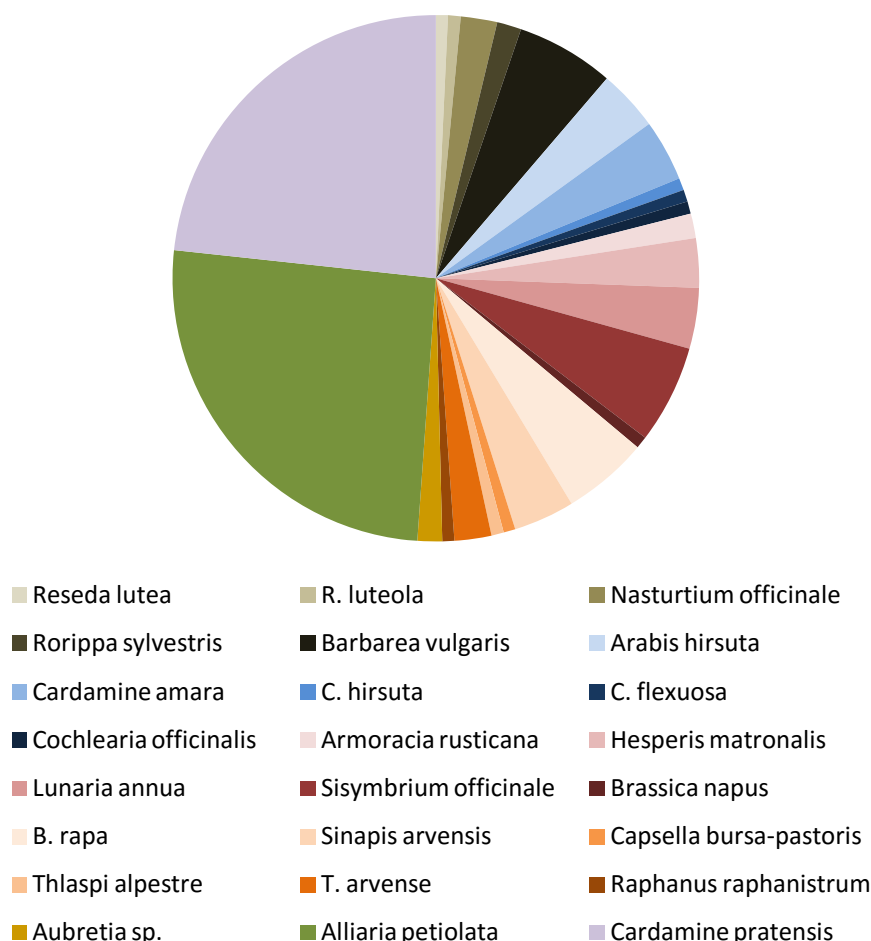


Fig. 1.13 *A. cardamines* host utilization in the British mainland. Most data are from records sent by 50 correspondents to Courtney and Duggan (1983), to which I have added Courtney's and my own observations. Each correspondent recorded which host plants were utilized in surveyed areas (total number of records = 133), but not the frequency of oviposition upon each host.

Overall, there were about an equal number of records from *A. petiolata* and *C. pratensis* (Fig. 1.13), but a change in utilization was detectable across the British mainland (Courtney and Duggan, 1983). In northern and western populations, *C. pratensis* was more often recorded as a host-plant than *A. petiolata*, whereas the converse was true in southern and eastern populations. Statistically, this difference amounted to a weak trend (Table 1.2), but when correspondents were asked to identify the major host being utilized, the difference between the two regions was

highly significant; *C. pratensis* is more frequently the major host in the north and west, and *A. petiolata* is always the major host in the south and east (Table 1.2).

Table 1.2 Changes in host utilization across the British mainland, from Courtney and Duggan (1983). The regional designations (e.g. "Rest of England") are taken directly from descriptions given in Courtney and Duggan (1983).

Criterion	Region	Ap	Cp	χ^2	p
Host use	Northern & Western populations	12	17	2.72	0.10
	Southern & Eastern populations	20	12		
Major host	Scotland, N & W England, Wales	6	9	11.49	0.0005*
	Rest of England	13	0		

Ap = *A. petiolata*, Cp = *C. pratensis*. * simulated p-value based on 2000 replicates

The change in host utilization is associated with changes in *A. cardamines*' population structure and habitat use across the British mainland (Courtney and Duggan, 1983). In the north, the butterfly tends to occur in small isolated populations, and is particularly associated with riverbanks and marshes (in some localities, e.g. Durham and Aberdeenshire, it is a riverbank specialist); whereas in the south, it occurs in loose open populations, such that nomadic specimens can be seen anywhere (outside densely urbanized areas). The shift from more specialized to more generalized habitat use (Fig. 1.14) reported by Courtney and Duggan (1983) is highly significant ($\chi^2 = 11.83$, simulated p-value (2000 replicates) = 0.0025). These authors hypothesize that the observed changes in habitat use and host utilization across the British mainland are connected; in particular, they suggest that the restriction of the butterfly to riverbank localities in Durham is an outcome of the scarcity of *A. petiolata* along hedges within that County. However, it has not been determined whether changes in host use are the result of changes in host abundance or host preference.

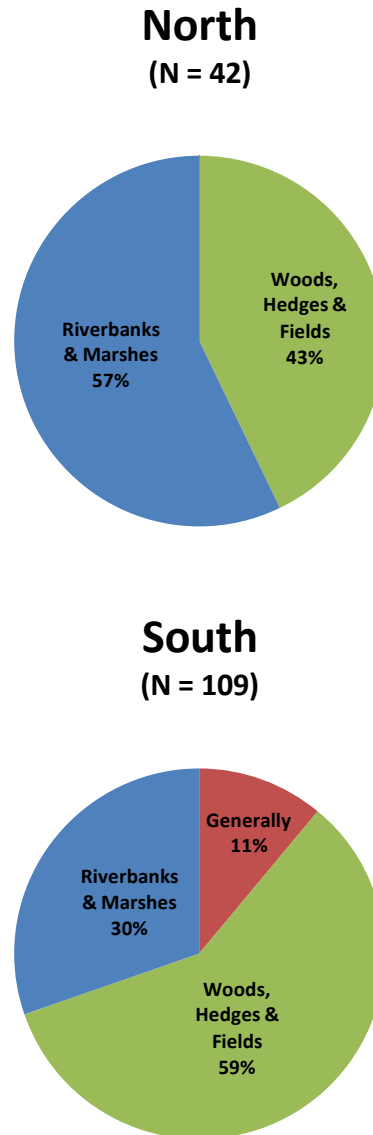


Figure 1.14 Changes in *A. cardamines* habitat use across the British mainland, from Courtney and Duggan (1983). The data for the "South" and "West" as defined by Courtney and Duggan have been combined into the "South" category here, since the difference between them was not significant ($\chi^2 = 1.39$, $df = 2$, $p = 0.5$).

A further dichotomy between northern and southern populations of *A. cardamines* in mainland Britain occurs with respect to the size of the imagines. From a study of specimens preserved in museum collections, Majerus (1979) found that the average wing-length of northern specimens is about 1 mm shorter than that of southern specimens (Table 1.3). This correlates well with the observed changes in host use, since Courtney (1981) found that pupae resulting from larvae bred on *C. pratensis* were less massive than those resulting from larvae bred on *A. petiolata*, and that pupal mass is correlated with wing-length. Specimens from Ireland and the Isle of

Man are even smaller than northern mainland specimens (Table 1.3), which again would correlate with host use, since in Ireland the "predominant" host is *C. pratensis* (Asher et al., 2001). However, Majerus (1979) found that size differences between specimens from Surrey and the Isle of Man were genetic (families originating from stock collected in the two localities retained the size differences when reared in a common environment), which suggests that regional differences in wing-length may not be entirely attributable to phenotypic plasticity. The most likely explanation is that geographical changes in host use have exerted a selection pressure on imaginal size, at least in Irish populations.

Table 1.3. Average wing-lengths of male and female *A. cardamines* museum specimens collected in different areas of the British Isles, from Majerus (1979). Majerus does not give standard errors, but the number of specimens measured is large (for the combined data from the three northern localities, number of males (N_m) = 107, number of females (N_f) = 64; from the three southern localities, N_m = 588, N_f = 313; and from Ireland and the Isle of Man, N_m = 99, N_f = 74).

Locality	Male Wing-length	Female Wing-length	Male Average	Female Average
Southern Scotland	20.2	21.8		
Cleveland, North Yorkshire	20.6	22.3	20.3	22.1
South Lancashire, Cheshire	20.1	22.0		
London, Surrey, Kent, Sussex	21.4	23.6		
Wiltshire, New Forest, Dorset	21.1	23.2	21.1	23.2
Devon, Cornwall	20.6	22.6		
Isle of Man	18.0	19.8		
Ireland	17.9	19.4	18.0	19.7

In summary, there has been a shift in *A. cardamines*' host use since the separation of the British Isles from Continental Europe 7000 years ago; whereas the Continental race is truly polyphagous, the British one has become largely restricted to the two host-plants *A. petiolata* and *C. pratensis*, although it still retains a degree of polyphagous behaviour. This change is associated with the fusion of two chromosomes, which possibly impacted the process. Within the British mainland, the major host-plant utilized switches from *A. petiolata* in the south to *C. pratensis* in the north, and this is correlated with a change in the average size of the imagines inhabiting these two regions, which is at least partly attributable to phenotypic plasticity. In Ireland the process of specialization appears to have gone one step further, since *A. cardamines* has shifted towards monophagy on *C. pratensis*, and the

smaller size of the imagines has a genetic basis (assuming the results obtained for the comparison between Isle of Man and Surrey specimens can be extended to Irish specimens, as seems likely).

If the geographical selection mosaic of the interaction between *A. cardamines* and its host-plants is to include hotspots of reciprocal selection, then there must be a two-way coevolutionary response affecting both trophic levels in at least some habitats. Very little evidence has so far been obtained as to whether any host-plants respond to selection pressures exerted by egg-laying females or mature larvae (which are pre-dispersal seed predators). In Sweden, *C. pratensis* plants from demes subjected to high *A. cardamines*' oviposition rates had higher levels of herbivory tolerance, as judged by seed production or the probability of flowering the year after attack, than plants from demes with low oviposition rates (Boalt et al., 2010; König et al., 2014). Moreover, polyploidization in *C. pratensis* affects its interaction with *A. cardamines* in that country: females prefer to oviposit on octaploids, but tetraploids suffer higher levels of attack at the population level due to their greater occurrence in sunny habitats (to which the butterfly is largely restricted), at least in some years (Arvanitis et al., 2007, 2008; König et al., 2015). These data suggest that the raw material on which reciprocal selection can act is available in the interaction between the butterfly and this host-plant species.

Strong and persistent selection pressures are likely imposed on some *A. cardamines* populations in Britain by the parasitic fly *P. vulgaris*. Collated records in the Tachinid Recording Scheme (<http://tachinidae.org.uk>) indicate a high frequency of attack across a wide range of British localities. In County Durham, Courtney and Duggan (1983) found that in one locality (Durham) the final infection rate (in pupae) was close to 40% in four successive seasons (Fig. 1.15); these data represent *underestimates*, since larvae were removed from the field before pupation and their exposure to the fly was curtailed. In a nearby locality (Wolsingham), however, the infection rate was consistently low and sometimes <10% (Fig. 1.15). In other localities (Witton Park and Croxdale) the infection rate varied widely among years (Fig. 1.15). Fluctuating intensity of selection within and among localities likely generate complex evolutionary dynamics across landscapes, leading to the formation of shifting geographical selection mosaics.

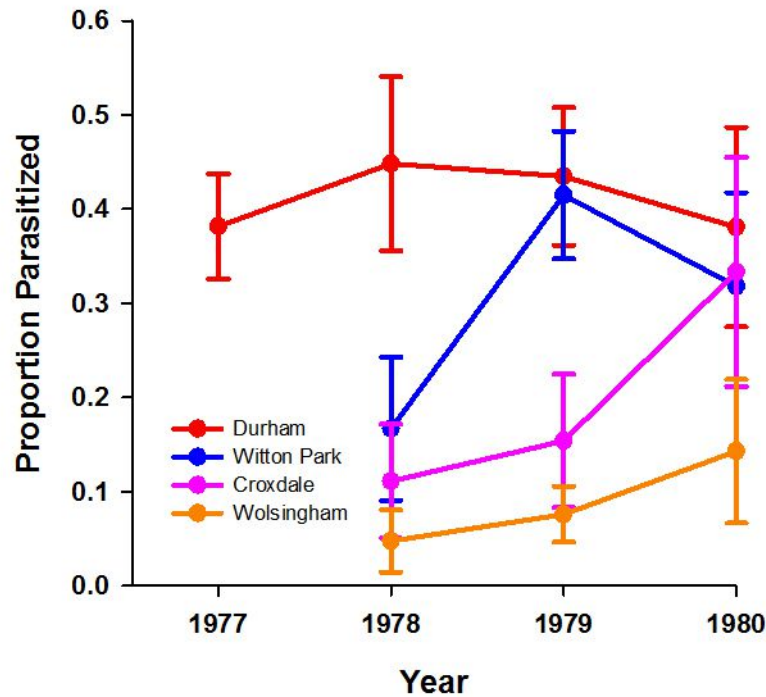


Figure 1.15. Proportion of *A. cardamines* larvae infected by *P. vulgaris* (\pm SE) in four localities in the Durham area, from Courtney and Duggan (1983).

Intuitively, the highly polyphagous behaviour of *P. vulgaris* suggests that its fitness will be relatively unaffected by any defences evolved by *A. cardamines* against it. However, circumstantial evidence indicates that this is not necessarily the case. The genus *Phryxe* contains five species in Britain, two of which are generalists and three of which are specialists, suggesting that speciation in this genus has repeatedly occurred through specialization to a narrower range of hosts. Among the two generalists, *P. nemea* has a tendency to favour *Abraxas grossulariata* and *P. vulgaris* a tendency to favour *A. cardamines*. It is not impossible therefore that cryptic speciation is occurring in these two species in relation to these interactions. In support of this conjecture, *P. vulgaris* larvae infecting *A. cardamines* have a tendency to aestivate and overwinter in the butterfly pupae (Courtney and Duggan, 1983); hence they do not emerge in August, which is the usual time of peak abundance for the adult flies (Belshaw, 1993), but in the following spring, when a new generation of *A. cardamines* larvae are available for attack. The synchronization of some strains of *P. vulgaris* with the univoltine life cycle of *A. cardamines* suggests they are specialized to it. In this case, geographical hotspots of reciprocal selection between the butterfly and the fly would be much more likely to occur.

In Sweden, *P. vulgaris* is replaced by the hymenopterous parasite *C. saltator*. This wasp can infect a very high proportion of *A. cardamines* larvae (Wiklund and Åhrberg, 1978; Wiklund and Friberg, 2009); the average over a five year period in Ljusterö was 66%, with the proportion varying between 55% and 84% (Fig. 1.16). Interestingly, *C. saltator* and *P. vulgaris* attack different larval stages; the former species infects the early instars (probably the first) and the latter the later ones (from the third instar onwards). This could modulate the coevolutionary response of the butterfly to the strong selection pressures exerted by them. In Sweden, between-plant, among-year, and among-site variation in mortality from *C. saltator* should select for ecological generalization in host use (Wiklund and Friberg, 2009), whereas in Britain, *P. vulgaris* could select for early termination of larval growth (this will be investigated in Chapter 4).

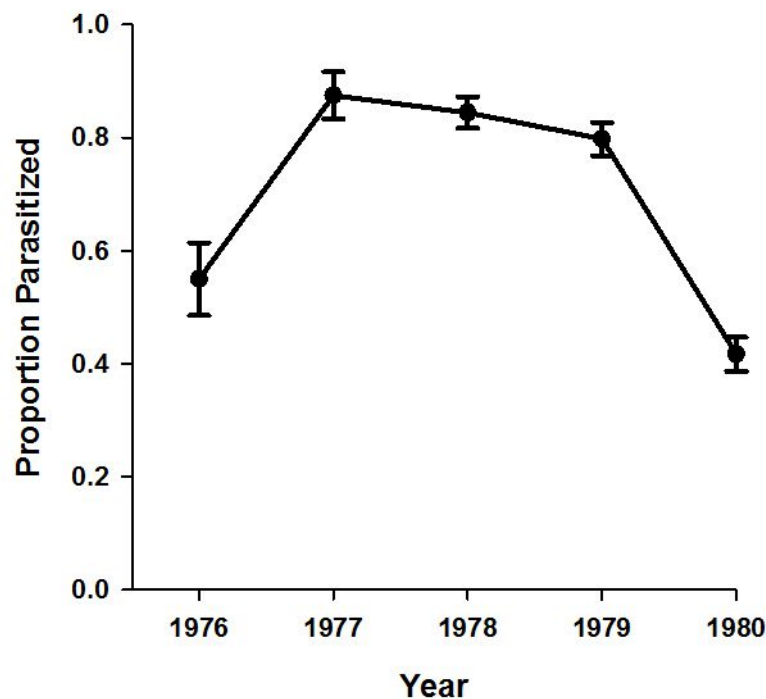


Figure 1.16 Proportion of *A. cardamines* larvae infected by *C. saltator* (\pm SE) in Ljusterö, Sweden, from Wiklund and Friberg (2009).

The tritrophic food web comprising the two endoparasites *C. saltator* and *P. vulgaris*, the herbivore *A. cardamines*, and the two autotrophs *A. petiolata* and *C. pratensis*, is therefore likely subject to genotype x genotype x environment interactions across landscapes, leading to the formation of geographic selection mosaics within which hotspots of reciprocal selection are possible. In addition to

selection mosaics and hotspots, Thompson (2005) identifies trait remixing via gene flow, random genetic drift and metapopulation dynamics as an important process shaping coevolutionary interactions. In northern Britain, small isolated populations of *A. cardamines* are likely subject to genetic drift and extinction, and vacated habitat patches to recolonization; Courtney (1980) obtained some evidence for genetic drift in small Durham populations, but could not rule out the possibility of local adaptation. Similarly, the restriction of *C. pratensis* to damp habitats should regularly expose it to the effects of isolation. Conversely, in southern England, the nomadic behaviour of *A. cardamines* and the widespread occurrence of *A. petiolata* under hedges and along roadsides should assist gene flow within these species across the landscape. These contrasting aspects of trait remixing could differentially affect the geographic mosaics in the north and south of the country.

Chapter 2

Effect of *Anthocharis cardamines* host-plant utilization on body-size, eclosion timing and dispersal

Abstract

*The evolution of host range and preference in phytophagous insects is driven by a female's oviposition choice impacting her offspring's fitness. Analysis of the fitness of progeny on different host-plants has commonly been restricted to the performance of immature stages. However, since host use can affect adult size, it is important to measure the ongoing effects of host choice on the resulting imagines. The orange-tip butterfly, *Anthocharis cardamines*, shows a strong preference for two host-plants in Britain, *Alliaria petiolata* and *Cardamine pratensis*, which affect body-size. Whilst females exhibit a strong positive size-fecundity relation, the impact of body-size variation is unknown in males. I here examine fitness effects of host-plant choice for male *A. cardamines*. Males reared on *C. pratensis* were smaller and emerged earlier than those reared on *A. petiolata*, and early-season males were smaller than late-season ones in the field. Interestingly, regression analysis indicated that the earlier emergence of small males was a host-mediated rather than a size-mediated effect. Small size was associated with reduced male dispersal in a semi-isolated wild population over a three year period. I propose that the earlier emergence associated with *C. pratensis* has evolved in response to depressed dispersal in isolated/semi-isolated populations associated with this patchily distributed host. I suggest that adult life-history traits are important for the maintenance of host range in this species, and offer a critique of Courtney's earlier hypothesis that host range is maintained by time-limited oviposition behaviour.*

[This Chapter formed the basis for the paper published by Davies and Saccheri, 2013]

Introduction

The evolution of host range and preference in phytophagous insects depends fundamentally upon host choice impacting on the reproductive success of ovipositing females (Jaenike, 1990; Thompson and Pellmyr, 1991; Mayhew, 1997; West and Cunningham, 2002; Agosta, 2008). Most attempts to find a direct relation between host rank and fitness have focused on larval performance, with mixed results (e.g. Courtney and Kibota, 1990; Mayhew, 1997, 2001; but see Gripenberg *et al.*, 2010). However, adult fitness effects cannot be neglected in an assessment of host quality (Awmack and Leather, 2002; De Block and Stoks, 2005; Moreau *et al.*, 2006; Moreau *et al.*, 2007; Agosta, 2010). In particular, since food quality and quantity can impact on adult size, the evolution of host preference may be associated with the

selection pressures acting on body-size. In butterflies, two adult life-history traits likely associated with body-size are eclosion timing and dispersal.

The expected trade-off between eclosion timing and body-size in relation to sexual size dimorphism was first pointed out by Singer (1982). Noting that protandry (prior emergence of males) must be limited to species exhibiting discrete generations, he hypothesized that selection for early male emergence would result in depressed male size (female-biased sexual size dimorphism), since advanced eclosion would require a shortening of the larval growth period. Strictly speaking, this hypothesis does not apply to species entering a long period of diapause in the pupal or imaginal stage before reproduction (Singer, 1982), since the postponement of the emergence period nullifies the selection pressure on larvae for early metamorphosis. However, Wiklund and Forsberg (1991) found that among winter diapausing butterflies (including *A. cardamines*), there was a positive relationship between protandry and small male size. This they attributed to smaller-sized male pupae requiring shorter post-diapause morphogenetic development times than larger-sized female pupae. If this is correct, then changes in body-size resulting from host use may impact emergence timing in adult butterflies.

In insects, changes in body-size are likely to be associated with changes in flight morphology. The effect of variation in flight morphology on dispersal has been studied in several butterfly and moth populations (Berwaerts *et al.*, 1998, Thomas *et al.*, 1998, Hill, Thomas & Blakeley, 1999, Hill, Thomas & Lewis, 1999, Kingsolver, 1999, Hanski *et al.*, 2002, Norberg & Leimar, 2002, Breuker *et al.*, 2007, Hughes *et al.*, 2007, Anderson *et al.*, 2008; Bridle *et al.*, 2014). In isolated populations, where the insects have had time to evolve locally, the flight morphology in both sexes may be adapted to reduce losses from, and hence increase the residence time in, the colonized area. Thus, Dempster *et al.* (1976) found that preserved specimens from the geographically isolated population of the swallow-tail butterfly (*Papilio machaon*) in Wicken Fen, Cambridgeshire, were smaller and possessed a different wing-morphology than those from the more extensive population in the Norfolk Broads; alterations in flight morphology consistent with reduced dispersal ability have also been demonstrated from museum specimens of the large blue butterfly (*Maculinea arion*) from isolated populations in Britain (Dempster, 1991).

Relatively few investigations have so far attempted to assess the relation between flight morphology and dispersal in the field directly by a mark-release-recapture (MRR) technique, relying instead on theoretical assumptions on how flight morphology will affect flight ability (e.g. Betts & Wootton, 1988, Berwaerts *et al.*, 2002). Moreover, the results which have been obtained using MRR so far have been inconsistent. Thus, Kingsolver (1999) found no difference in survival-rate between either naturally occurring or experimentally manipulated size variants of *Pontia occidentalis*; similarly, Hanski *et al.* (2002) found that migration rate was not correlated with body size measurements in *Melitaea cinxia*. However, Kuussaari *et al.* (1996) found that migratory female *M. cinxia* were larger (as measured by forewing length) than non-migratory ones, and Breuker *et al.* (2007) showed that dispersive and non-dispersive females of this species differed in forewing shape. Van Dyck *et al.* (1997) also found indications for greater mobility in large (forewing length) male *Pararge aegeria*, although this effect could not be separated from the influence of their darker colouration impacting upon thermoregulation.

In this Chapter, I investigate whether host use impacts body-size in *A. cardamines* (in the laboratory and in the field), as predicted by Courtney's (1981) data on pupal mass, and whether this in turn impacts male eclosion timing (in the laboratory) and dispersal (in the field). From Courtney's data, it is predicted that specimens utilizing *A. petiolata* will be larger than those utilizing *C. pratensis*, and from the results summarized above, it is predicted that larger males will emerge later and disperse more rapidly than smaller ones. This would provide direct evidence that host use affects important adult life history traits in *A. cardamines* males.

Methods

Effect of host-plant on body-size

A. cardamines larvae (N = 96) were located on different host-plants (*A. petiolata*, "small" and "large" *C. pratensis*, and *B. vulgaris*) in Dibbinsdale Nature Reserve and were measured daily through the fifth instar. Body-length (from the tip of the head capsule to the tip of the anal flap) was chosen as a suitable metric of body-size; since the final size of the larva is largely determined by the amount of feeding in the fifth instar, head capsule width would not have been accurate, whereas an assessment of pupal mass would have required the removal of a prohibitively large number of

specimens from the Reserve. After a specimen vacated its host, the plant was revisited for three more days, since larvae can sometimes be missed. The maximum length in the sequence of daily measurements was the datum used for each specimen.

For adults, wing-length was used as a metric of body-size, since it is easily obtained from living specimens in the field. To prove and calibrate the relationship between wing-length and larval body-length, these measurements were obtained from 102 specimens (48 males, 54 females) reared under common garden conditions from stock descended from larvae collected outside (but near to) the Reserve. All measurements on living specimens were made to the nearest mm with a 15 cm rule; wing-length measurements for the common garden experiment were made after death on detached wings to the nearest 0.01 mm using digital callipers.

Timing of eclosion

Larvae were reared on *C. pratensis* and *A. petiolata* in common garden conditions (on potted plants at room temperature in the early instars, then on plant cuttings in yoghurt pots at room temperature in the late instars), and following pupation were kept in a common environment (an indoor room during the summer months, an outhouse during the winter months) until emergence. During the eclosion period, pupae were transferred to individual collecting boxes and the emergence day of each specimen recorded, with day 1 being the day on which the first specimen eclosed.

The effect of host-plant on eclosion time in nature was inferred from intra-seasonal changes in wing-length. For each year in the study period, early/late season specimens were respectively defined as those captured before/after the date on which the cumulative number of specimens encountered that year reached half the eventual total number; specimens encountered *on* this date were assigned to either early or late season according to which allocation best equalized numbers in the two half-season samples.

Dispersal-rate

A mark-release-recapture technique was used to analyze the dispersive behaviour of the butterflies over a three year study period (2005-2007). On first capture, specimens were immobilised between the folds of a butterfly net and wing-length measured to the nearest millimetre on the underside of the left forewing from the

base of the costa to the apex. The specimens were removed from the net with entomological forceps and marked with a coded number in the area of the orange-tip markings on the undersides of the forewings. These are concealed beneath the hindwings when the specimens are at rest, so the natural cryptic colouration of the undersides is not interfered with when the wings are marked there. The specimens were transferred to collecting boxes and kept in the dark until release. All specimens were released in the vicinity where they were captured (or recaptured); none were observed to undertake an escape flight upon release.

Sampling was largely restricted to seven sub-sites (here termed the sub-sites of first capture, or SSFC) within the Reserve (or whole-site, WS), containing large patches of *C. pratensis* or *A. petiolata* (Fig. 1.5; see *Introduction* for more details). As well as being the principal hosts, these plants are often utilized as nectar sources by the imagines; the patches were regularly visited by the males. Each patch was separated from its nearest neighbour by distance or, in one case, a railway track (Fig. 1.5).

Residence plots (termed recapture-duration decay-plots by Watt *et al.* (1977)) for the WS were constructed from the natural logarithm of the number of specimens remaining in the Reserve against the time elapsed since first capture; a specimen was regarded as present in the reserve every day until the day after its last capture. Similar plots were constructed from the combined data for the SSFC, to which specimens were regarded as confined while they were *exclusively* captured in them; although this does not necessarily imply their true confinement to them, it can be taken as indicative of an initially restricted flight range within the Reserve. Once a specimen was captured outside its SSFC, it was taken to have emigrated from it, even if it subsequently returned there; the day after its last capture was taken to be the day on which it emigrated from it. In both cases, the elapsed time does *not* include poor weather days on which the insects were immobile, since their inclusion was found to markedly disrupt the decay-plots.

Separate decay-plots were constructed for small (15-18 mm), medium (19-20 mm) and large (21-22 mm) sized specimens (these classes cover the range of male wing-lengths and were chosen so that males of average wing-length are medium sized); best-fit lines were obtained using least squares linear regression. Data relating to the two spatial scales (SSFC and WS) and three years of the study period (2005-2007)

were plotted separately, and an F test used to determine whether there were significant differences among the decay-plot gradients for the different sized butterflies; if so Tukey tests were used to determine which gradients were distinct (Zar, 2010). Since there were no marginal cases with respect to significance/non-significance, data from size-groups which did not differ significantly were combined. The gradients (m) of these plots were then used to determine the *daily residence-rates* ($= e^m$) of the different sized butterflies, where ‘residence-rate’ is understood to be influenced by both emigration and death (Watt *et al.*, 1977).

To unravel the dispersive behaviour of the butterflies, the WS decay-plots were restricted to those specimens which were recaptured at least once in the SSFC. This enabled the *daily resettlement-rate* (r), defined as the daily probability that a living specimen will leave its SSFC *and* resettle elsewhere in the WS, to be calculated as follows. Let N be the total number of specimens present in all the SSFC, and hence in the WS, on day i . Then on the next day, $i + 1$, there will be N_s specimens left in the SSFC and N_w in the WS, where s and w are the daily residence-rates of specimens in the SSFC and WS respectively. Therefore the daily proportion of specimens which transit from the SSFC to the WS is $(N_w - N_s)/N$, or

$$r = w - s \quad (2.1)$$

Under certain conditions, the resettlement-rate allows emigration to be distinguished from death (see Appendix 1).

Statistical analyses

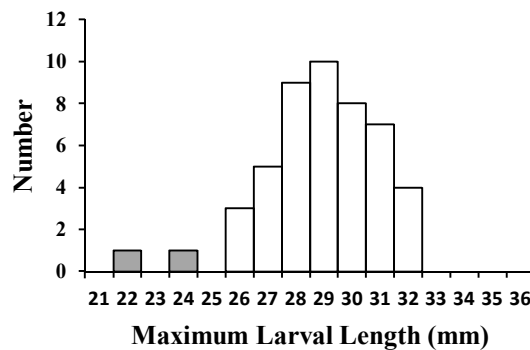
All means are quoted with 95% C.I. When means were compared (z-test, t-test, ANOVA), the data were checked for equality of variances (Levene’s test) first. For the two-way ANOVA examining the effects of season and year on wing-length (Table 2.4), the analysis was re-run with wing-length values raised to the fifth power, which eliminated the negative skewing in the original wing-length distributions. Since this resulted in almost identical results to those obtained without data transformation, and since ANOVA is known to be robust to departures from normality (Zar, 2010), I here report the results obtained from the untransformed data.

Results

Host-plant use and size variation

Wild final instar *A. cardamines* larvae attained a significantly shorter average body-length on small *C. pratensis* than they did on large *C. pratensis*, *A. petiolata* and *B. vulgaris* ($z = 4.96$, $p < 0.0001$) (Fig. 2.1). A few dwarf larvae (21-24 mm) were recorded from both small *C. pratensis* and the larger plants; these were excluded from the calculation for the z-test. It is possible that these specimens were lost to predation before attaining full size, but such dwarfs have been observed to pupate in captivity (Fig. 2.2a).

(a) Small *C. pratensis*



(b) Large *C. pratensis*,
A. petiolata, *B. vulgaris*

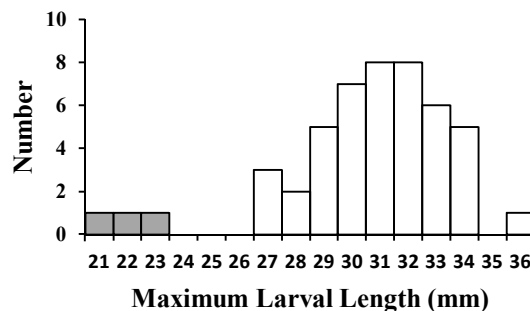
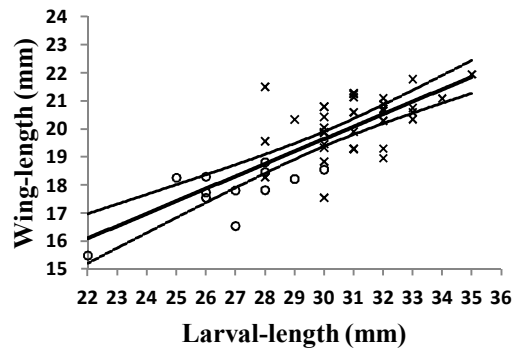


Figure 2.1 *A. cardamines* final larval body-length distributions on different sized host-plants in Dibbinsdale Nature Reserve. Specimens considered outliers are shaded in grey. (a) Larvae on small *C. pratensis* (average plant-height < 30 cm), mean (excluding outliers) = 29.1 ± 0.5 mm ($N = 46$). (b) Larvae on large *C. pratensis*, *A. petiolata* and *B. vulgaris* (average plant-height > 30 cm), mean (excluding outliers) = 31.1 ± 0.6 mm ($N = 45$).

The relationship between imaginal wing-length and larval body-length, as determined from least squares regression analysis on a sample of 48 males reared

under control conditions (Fig. 2.2a), was $W = 0.44L + 6.34$, where W is the wing-length of the imagines and L the body-length of their final instar larvae. The slope of the regression line is highly significant ($t_{46} = 8.49$, $p < 0.0001$). (A similar relationship was obtained for the females (Fig. 2.2b), with $W = 0.44L + 7.02$ ($t_{52} = 7.57$, $p < 0.0001$)). Valid deductions concerning the wing-length of *A. cardamines* adults can be made, therefore, from the body-length of their larvae. For both sexes, specimens raised on *C. pratensis* were smaller (as both larvae and adults) than those reared on *A. petiolata* (Fig. 2.2), in line with the results obtained in the field.

(a) Males



(b) Females

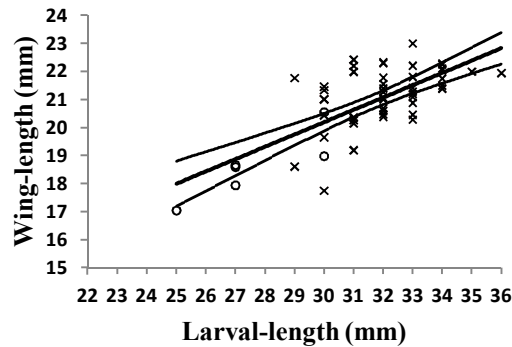


Figure 2.2 Regression (with 95% confidence bands) of *A. cardamines* wing-length (W) against larval body-length (L) for (a) males ($W = 0.44L + 6.34$, $R^2 = 0.61$, $p < 0.0001$, $N = 48$) and (b) females ($W = 0.44L + 7.02$, $R^2 = 0.52$, $p < 0.0001$, $N = 54$). Specimens reared in common garden conditions on small *C. pratensis* and *A. petiolata* are represented by circles and crosses, respectively. Note that a 22 mm (body-length) male larva successfully pupated, producing a 15.50 mm (wing-length) adult.

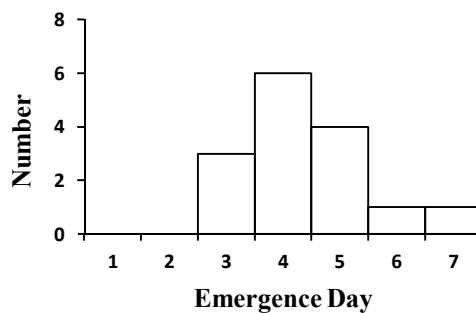
Eclosion time

Males emerged significantly earlier when reared on small *C. pratensis* than they did when reared on *A. petiolata* ($t_{51} = 2.15$, $p = 0.036$) (Fig. 2.3). The eclosion window

was much narrower in captivity (seven days) than in the field (six to seven weeks), perhaps because the pupae had been kept in uniform conditions. The different emergence patterns among host-plants can be attributed to differences in post-diapause development rate, since there was no correlation between pupation date and eclosion date ($r = 0.16$, $df = 44$, $p = 0.14$ (one-tailed)) (Fig. 2.4). (There was also no correlation for females: $r = 0.03$, $df = 51$, $p = 0.42$ (Fig. 2.4)).

From the data for five families whose broods were split between the two hosts, a univariate linear model (Table 2.1) reveals significant effects on eclosion time of both family and host. Hence, the effect of the host-plants on eclosion time is still significant when family (presumably genetic) effects are controlled for. It was not possible to test for interaction directly, since the error variances were inhomogeneous for a model including an interaction term, but since the mean emergence times of specimens reared on small *C. pratensis* were earlier than for those reared on *A. petiolata* in every family, any interaction between family and host was limited.

(a) Small *C. pratensis*



(b) *A. petiolata*

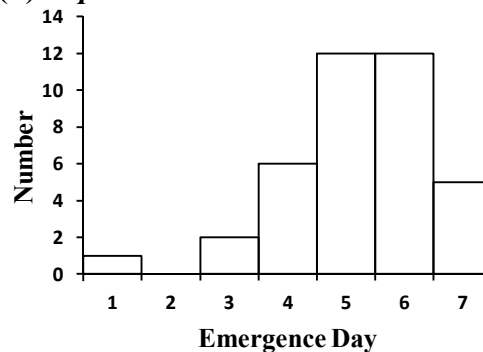


Figure 2.3 Emergence patterns of male *A. cardamines* reared on (a) small *C. pratensis*, mean \pm 95% CI = 4.4 ± 0.6 d ($N = 15$), and (b) *A. petiolata*, mean \pm 95% CI = 5.2 ± 0.4 d ($N = 38$).

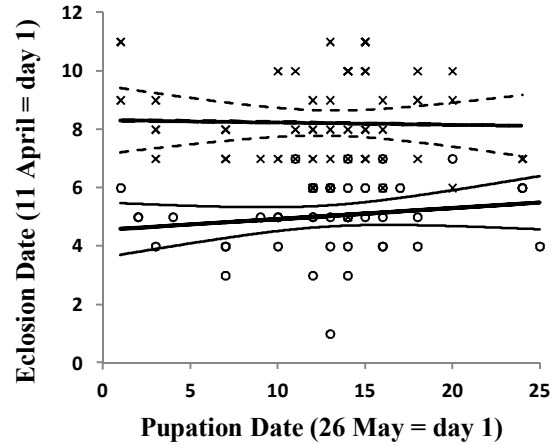


Figure 2.4 Regression (with 95% confidence bands) between eclosion date (E) and previous years' pupation date (P) in male (circles, solid line) and female (crosses, dashed line) *A. cardamines*. Neither relationship is significant (for males: $E = 0.038P + 4.54$, $R^2 = 0.027$, $p = 0.28$, $N = 46$; for females: $E = -0.008P + 8.31$, $R^2 = 0.001$, $p = 0.85$, $N = 53$).

Table 2.1 A univariate general linear model for the effects of host-plant and family on eclosion time of male *A. cardamines* in captivity.

Source	Type III SS	df	MS	F	p
Corrected Model	21.485	5	4.297	6.997	<.001
Intercept	641.357	1	641.357	1044.299	<.000
Host	4.464	1	4.464	7.268	.012
Family	16.684	4	4.171	6.791	.001
Error	15.354	25	.614		
Total	753.000	31			
Corrected Total	36.839	30			

Interestingly, regression analysis showed that once the effect of host use had been controlled for, there was no significant relation between body-size and eclosion time among males (Table 2.2). Moreover, females bred on *C. pratensis* which were significantly *smaller* ($t_{41} = 3.55$, $p < 0.001$) than males bred on *A. petiolata* emerged significantly *later* ($t_{41} = 5.216$, $p < 0.0001$) than them (Table 2.3).

Table 2.2. Regression analysis on the effects of host-plant and wing-length on eclosion day for male *A. cardamines* in captivity.

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations		
	B	S.E.	Beta			Zero-order	Partial	Part
(Constant)	6.090	2.715		2.243	.030			
Host-plant	1.236	0.588	0.438	2.102	.041	.289	0.293	0.291
Wing-length	-0.165	0.173	-0.199	-0.955	.345	.129	-0.138	-0.132

Dependent Variable: Eclosion Day

Table 2.3. Variation in *A. cardamines* wing-length and eclosion-time with sex and host-plant. The mean eclosion day for males bred on *C. pratensis* is different to that quoted in Fig. 2.3 because two males were crippled on emergence and their wing-lengths could not be measured.

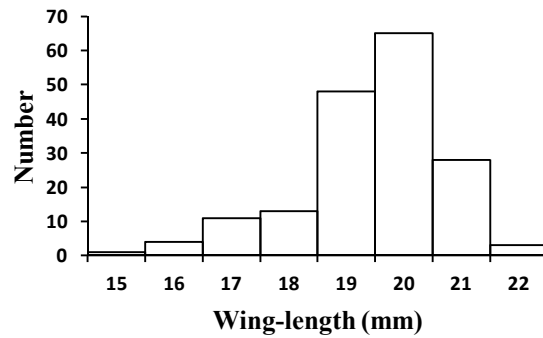
Sex	Host-plant	N	Mean Wing-length (mm)	Mean Eclosion Day
Males	<i>C. pratensis</i>	13	17.59	4.62
	<i>A. petiolata</i>	37	20.22	5.22
Females	<i>C. pratensis</i>	6	18.63	8.33
	<i>A. petiolata</i>	48	21.19	8.21

In the field, early emerging male *A. cardamines* were significantly smaller on average than those emerging in late season over the three years of the study period, with no detectable size difference between years or difference in seasonal effect between years (Table 2.4; Fig. 2.5). The effect on the proportion of small and large specimens was very strong at particular sub-sites in some seasons (Fig. 2.6). Taken together with the known depression of body-size and earlier eclosion in captivity associated with small *C. pratensis*, these results indicate that host-plant utilization affects the timing of eclosion in male *A. cardamines* in nature.

Table 2.4. A general linear model for the effects of year and season (early or late) on the size (wing-length) of male *A. cardamines* in Dibbinsdale for 2005-2007.

Source	Type III SS	df	MS	F	p
Corrected Model	19.031	5	3.806	2.479	.032
Intercept	127162.009	1	127162.009	82807.102	<.001
Year	.843	2	.422	.274	.760
Season	17.044	1	17.044	11.099	.001
Year * Season	.350	2	.175	.114	.892
Error	503.690	328	1.536		
Total	129877.000	334			
Corrected Total	522.722	333			

(a) Early Season



(b) Late Season

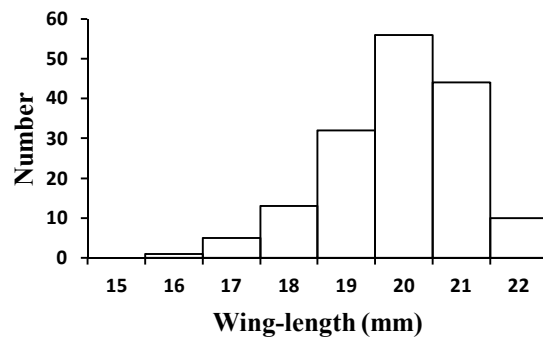
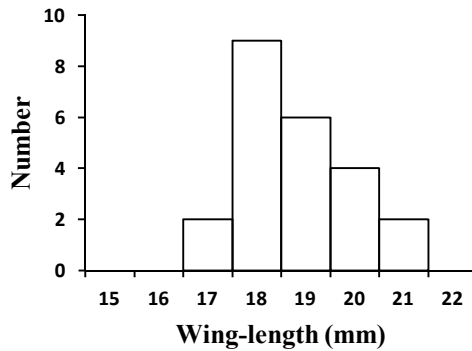


Figure 2.5. Intra-seasonal size variation in male *A. cardamines* in Dibbinsdale, from the combined data for 2005-2007; (a) early season, mean \pm 95% CI = 19.46 ± 0.19 mm, N = 173, (b) late season, mean \pm 95% CI = 19.92 ± 0.19 mm, N = 161. Numbers in the early and late season samples for each year were equalized as far as possible as described in *Methods*.

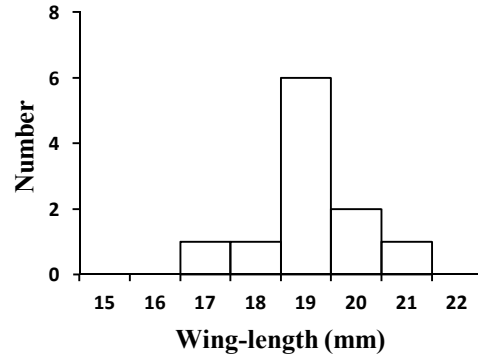
Size-dependent dispersal

In every year of the study period, there were size related differences in the *daily residence-rate* of male *A. cardamines* in the field, as determined from the gradients (Table 2.5) of their residence plots (Fig. 2.7). The spatial scale on which these differences could be detected, as well as the size categories involved, varied among years, but in general smaller specimens tended to exhibit higher residence-rates than larger ones.

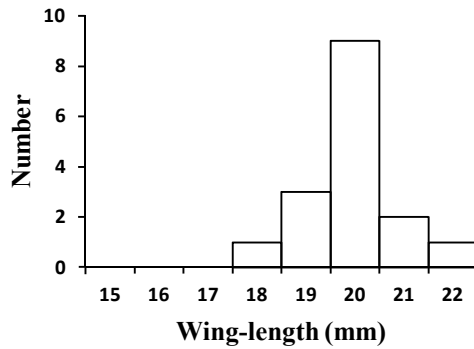
(a) OT + UT (26/4)



(b) BH (24/4)



(c) OT + UT (13/5)



(d) BH (13/5)

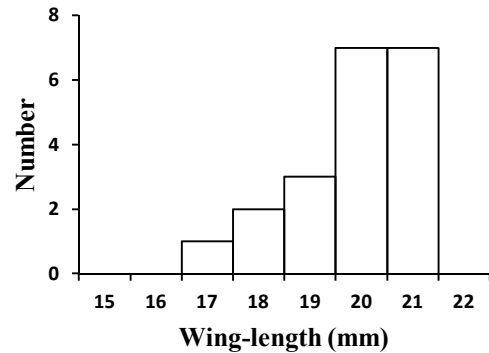


Figure 2.6 Intra-seasonal changes in male *A. cardamines* wing-length distributions at specific sub-sites in 2004. (a) Otters' Tunnel + Upper Tip early season, mean \pm 95% CI = 18.78 ± 0.49 mm (N = 23), (b) Boden's Hey early season, mean \pm 95% CI = 19.09 ± 0.70 mm (N = 11), (c) Otters' Tunnel + Upper Tip late season, mean \pm 95% CI = 19.94 ± 0.49 mm (N = 16), (d) Boden's Hey late season, mean \pm 95% CI = 19.85 ± 0.55 mm (N = 20). (Lower Tip was not visited in 2004, so combined data from OT and UT are for a contiguous area in the Reserve). Figures in parentheses are average dates (day/month) of first capture of sampled specimens. For the comparison between the proportion of early and late season specimens measuring <20 mm or ≥ 20 mm at OT + UT, $\chi^2 = 9.08$, df = 1, p = 0.003; at BH, $\chi^2 = 5.23$, p = 0.028 (simulated p-value based on 2000 replicates).

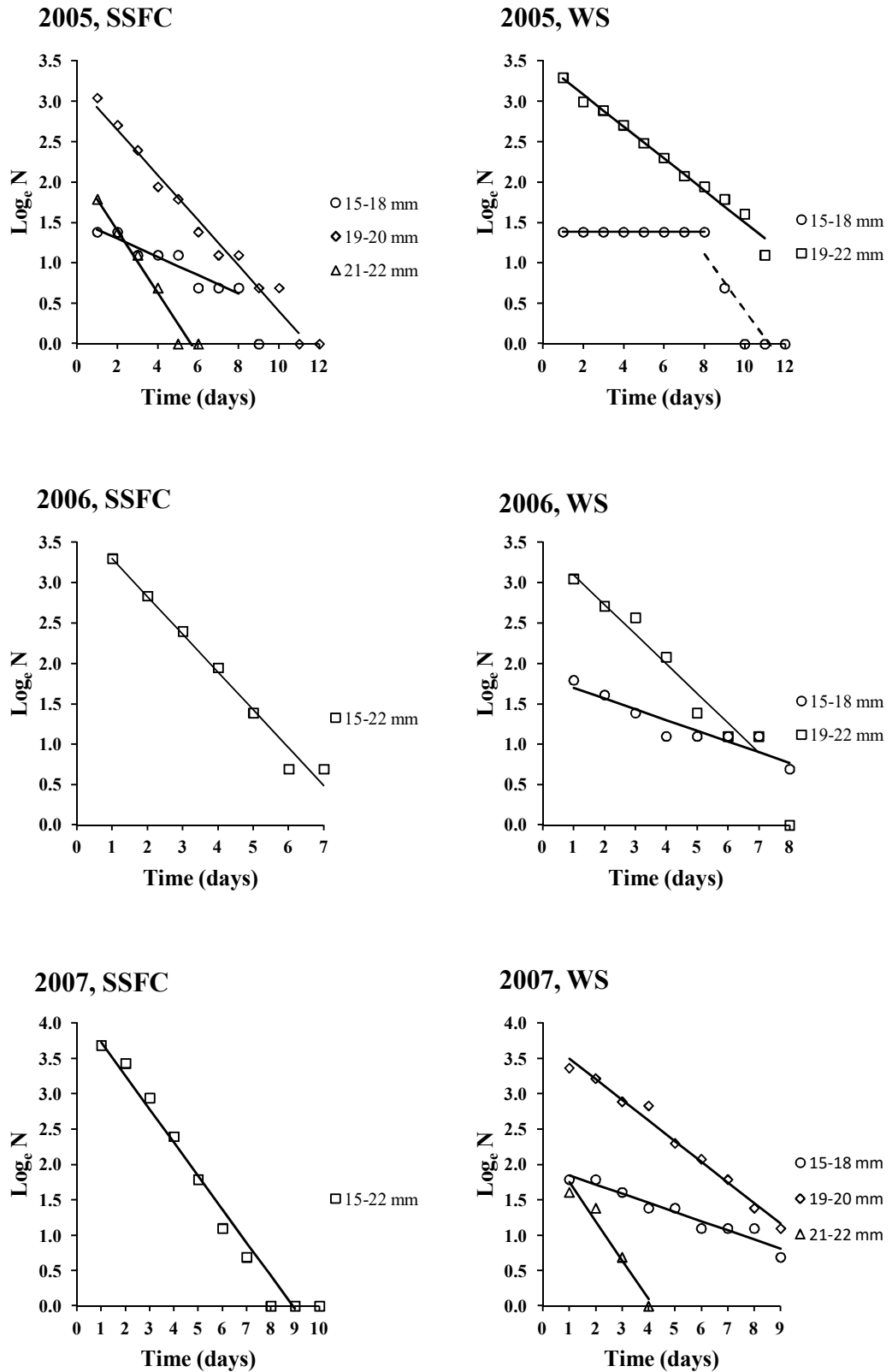


Figure 2.7 Residence plots on two spatial scales for different sized male *A. cardamines* in Dibbinsdale for each year of the study period. Wing-length classes were combined whenever the gradients of their best-fit lines did not differ significantly; the dashed line shows the presumed senescent period of the small specimens in 2005 (WS).

Table 2.5 The gradient (m), standard error (SE) and degrees of freedom (df) for the best-fit lines of the decay-plots (Fig. 2.7) for male *A. cardamines* in different size classes at two spatial scales (sub-site of first capture, SSFC, and whole site, WS) in Dibbinsdale for 2005-2007. (Data have been combined for wing-length classes which did not differ significantly.)

Year	Spatial Scale	Wing-length	m	SE	df
2005	SSFC	15-18 mm	-0.1135	0.0162	6
		19-20 mm	-0.2799	0.0141	9
		21-22 mm	-0.3864	0.0360	4
	WS	15-18 mm	0.0000*	0.0000	5
			-0.3466†	0.1059	3
		19-22 mm	-0.1978	0.0086	9
2006	SSFC	15-22 mm	-0.4679	0.0289	5
	WS	15-18 mm	-0.1322	0.0206	6
		19-22 mm	-0.3656	0.0357	6
2007	SSFC	15-22 mm	-0.4713	0.0310	8
	WS	15-18 mm	-0.1297	0.0127	7
		19-20 mm	-0.2921	0.0134	7
		21-22 mm	-0.5522	0.0814	2

* pre-senescent phase, † post-senescent phase

It can be shown (Appendix 1) that the smaller specimens were *dispersing* more slowly from the WS than the large ones if three conditions hold.

1. That the SSFC residence-rates (s) are the same for the small and large butterflies.
2. That the *daily resettlement-rate* of the small specimens is higher than that of the large ones.
3. That the small specimens do *not* disperse more quickly from their SSFC than the large ones.

The daily resettlement-rates of the different sized butterflies in the three years of the study period are shown in Table 2.6. In 2006, small (15-18 mm) specimens exhibited higher resettlement rates than medium (19-20 mm) or large (21-22 mm) sized ones, and in 2007 there was an inverse relationship between the size of the butterflies and their resettlement-rate. Since in both these years s was constant, the first two conditions are met. Data bearing on the third condition is given in Table 2.7, which shows that among specimens which remained alive and in the WS long enough to be recaptured at least once, the smaller ones were more likely to be recaptured in their SSFC; when the numbers in the small and medium size classes are combined, this

difference is significant ($\chi^2 = 4.30$, $df = 1$, $p = 0.038$). This suggests that the smaller butterflies were less dispersive on this scale than the large ones; more conservatively, it may be assumed that the small specimens did *not* disperse more quickly than the large ones from their SSFC, and so the third condition is also met. Hence smaller specimens were emigrating from the WS more slowly than large ones in 2006 and 2007. It is likely that a similar conclusion applies to both the SSFC and WS in 2005, but this cannot be shown directly.

Table 2.6 Dispersive behaviour of different sized male *A. cardamines* in Dibbinsdale for the years 2005-2007. The daily residence-rate for specimens in the WS (*w*) and SSFC (*s*) were derived from the gradients given in Table 2.5. The *daily resettlement rate* was calculated from equation 2.1.

Year	Wing-length	<i>w</i>	<i>s</i>	Daily Resettlement Rate
2005	15-18 mm	1.00	0.89	0.11
	19-20 mm	0.82	0.76	0.06
	21-22 mm	0.82	0.68	0.14
2006	15-18 mm	0.88	0.63	0.25
	19-20 mm	0.69	0.63	0.06
	21-22 mm	0.69	0.63	0.06
2007	15-18 mm	0.88	0.62	0.26
	19-20 mm	0.75	0.62	0.13
	21-22 mm	0.58	0.62	(-0.04)

Table 2.7 The numbers (N) and percentages (%) of male *A. cardamines* in each wing-length category which were recaptured within or outside their SSFC in Dibbinsdale in 2005-2007.

	15-18 mm		19-20 mm		21-22 mm	
	N	%	N	%	N	%
Total recaptured	22	-	99	-	38	-
Total recaptured in SSFC	16	73	64	65	18	47
Total recaptured outside SSFC	6	27	35	35	20	53

Discussion

In male *A. cardamines*, two key life-history traits, dispersal-rate and eclosion time, are likely to be related to host use. Smaller butterflies tend to have longer residence times in the field, and since host use impacts on larval size (in the field) and imaginal size (in captivity), it most likely affects dispersal-rate. Specimens reared on small *C. pratensis* emerged earlier than those reared on *A. petiolata*. These data, as well as the size-fecundity relation for female *A. cardamines*, add to the growing body of

evidence that host use has important consequences for *adult* life-history traits. It is therefore important to include an assessment of these traits in host preference-performance relationships, which have so far tended to focus on larval survival.

Singer (1982) pointed out that selection for protandry in insects can only occur in species with discrete generations, and proposed that this would likely result in small male size due to a trade-off between size and development-time. Wiklund and Forsberg (1991) found no evidence for such a trade-off in directly developing generations of European pierid and satyrid butterflies, but they did find a relationship between the degree of sexual size dimorphism and protandry in diapausing generations. This led them to propose that post-diapause morphogenetic development times are a function of pupal mass, and hence are shorter for smaller-sized male pupae. However, since small females (reared on *C. pratensis*) emerged later than large males (reared on *A. petiolata*) in my experiments (Table 2.3), this hypothesis can be rejected for *A. cardamines*.

Among *A. cardamines* males, the earlier emergence of small specimens in nature is a host-mediated rather than a size-mediated effect, since in captivity the precocious eclosion of specimens bred on small *C. pratensis* cannot be attributed to their reduced size (Table 2.2). The depressed dispersal of smaller males in nature probably is a size-mediated effect, however, since they are less robust than the larger specimens. I propose that the coupling between early emergence and small size in male *A. cardamines* is better interpreted as a coupling between early emergence and depressed dispersal, and suggest that this may have evolved as an “emerge early and wait” strategy associated with small *C. pratensis*. This host-plant is patchily distributed across landscapes, but where it occurs it is frequently abundant. Hence, male orange-tip butterflies which have utilized it will regularly emerge in localized habitat patches with a high density of conspecifics. This will simultaneously select against dispersal (due to the high density of locally emerging females) and increase competition for mates (due to the high density of locally emerging males). In game-theoretic models of emergence timing, the evolutionarily stable degree of protandry increases with survival-rate and population density (Zonneveld and Metz, 1991). If dispersal significantly depresses fitness, it will effectively increase the death-rate; conversely, philopatry will increase the effective survival-rate. In these circumstances, selection for depressed dispersal and early emergence will be tightly

coupled (Appendix 2). For example, if the WS residence-rates (w in Table 2.6) for different sized males in 2007 (which vary due to differences in dispersal-rate) are equated to the survival-rate in the Parker-Courtney model of protandry (Parker and Courtney, 1983) (to be described in the next Chapter), then the resulting ESS (evolutionary stable strategy) emergence curves (which describe the emergence schedule obtained when all males have equal fitness and gain 1 mating) confirm that smaller, more sedentary males should be selected to emerge earlier than larger, more dispersive ones (Fig. 2.8).

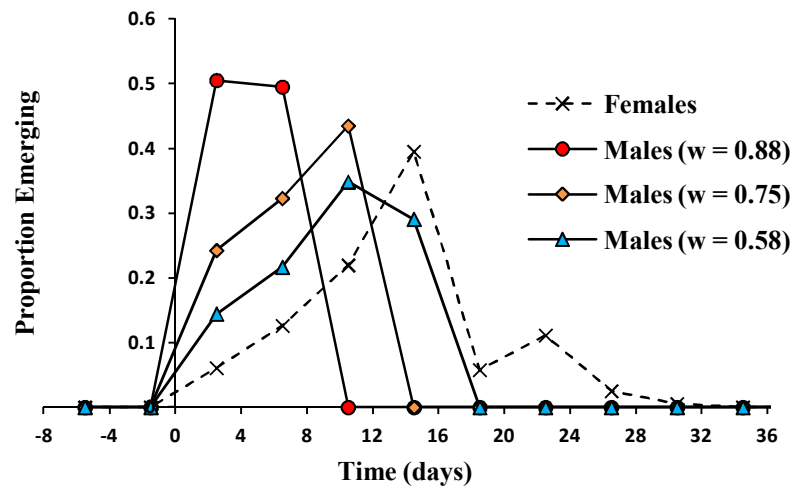


Figure 2.8 ESS emergence curves for different sized (wing-length) males (15-18 mm, red circles; 19-20 mm, orange diamonds; 21-22 mm, blue triangles) in 2007, calculated from the observed female emergence curve that year (grey crosses) and the Parker and Courtney (1983) simulation model, on the assumption that their residence-rates (w) can be equated to the survival-rate in the model.

In contrast to *C. pratensis*, *A. petiolata* tends to be more thinly and continuously distributed across landscapes, due to its occurrence under hedges along road verges. The low average population density encountered by *A. cardamines* males emerging in these situations will reduce the evolutionarily stable degree of protandry (Zonneveld and Metz, 1993), while rapid dispersal will increase the efficiency with which widely separated females are located. Hence, the utilization of *A. petiolata* should favour an "emerge late and rove" strategy, leading to selection for reduced protandry and large body-size.

In summary, the contrasting distribution patterns of *C. pratensis* and *A. petiolata* across landscapes is hypothesized to have selected for different adult life-history traits in male *A. cardamines*. This is effected through phenotypic plasticity, since

individuals in the same family can switch between responses depending on host-plant (Table 2.1). Since the two hosts have overlapping niches, they will occur together in some localities, as they do in Dibbinsdale. This will present a problem for males utilizing *A. petiolata*, since they will emerge consistently later than those utilizing *C. pratensis* in dense populations where both hosts occur, and so will be at a disadvantage. In fact, selection for early emergence of small, slow dispersing males utilizing *C. pratensis* may be assisted in high density populations by competition with large, fast dispersing ones utilizing *A. petiolata* (Appendix 2); if such populations are isolated, this would lead to monophagy on small *C. pratensis* in the absence of additional selection pressures (Appendix 2). Interestingly, in northern Britain, where populations are more isolated, the more favoured host-plant is *C. pratensis*. It is possible that in an area where climatic conditions are poorer, roving males searching for widely scattered females are disadvantaged by time constraints; if so, depressed dispersal (philopatry) will be favoured in core populations and this could have selected for an increased preference for *C. pratensis*. In southern Britain, where climatic conditions are milder, there will likely be considerable movement of individuals between compact core and loose open populations, maintaining oviposition on both hosts.

The occurrence of additional selection pressures acting on host preference is indicated by the fact that in northern populations *A. petiolata* is still utilized, and in southern ones it is more favoured than *C. pratensis* (Courtney and Duggan, 1983). The strong size-fecundity relationship for female *A. cardamines* indicates that more fecund females will be produced on *A. petiolata*. Hence, even in isolated northern populations, oviposition on both hosts could be maintained by a sexually-antagonistic trade-off between depressed dispersal (benefiting males and favouring oviposition on *C. pratensis*) and increased fecundity (benefiting females and favouring oviposition on *A. petiolata*). In open southern populations, the balance may tipped further in the direction of *A. petiolata*. Wiklund and Friberg (2009) found evidence that in Swedish populations of *A. cardamines*, polyphagy is maintained by a combination of host rarity, host unreliability (among year variability in availability) and host inconstancy (among year variability in suitability). Courtney (1982b) argued that the latter two factors are unlikely to be important in British populations; even if all three factors occasionally operate (e.g. Dempster (1997) showed that the

availability of *C. pratensis* in Monks Wood, southern England, varied considerably among years), they do not preclude adult life history traits from potentially impacting the evolution of host use. (For a critique of Courtney's (1982b) hypothesis on the maintenance of polyphagy in *A. cardamines*, see Appendix 3).

Chapter 3

Male emergence schedule and dispersal behaviour are modified by mate availability in heterogeneous landscapes

Abstract

*Protandry (prior emergence of males) in insect populations is usually considered to be the result of natural selection acting directly on eclosion timing. When females are monandrous (mate once), males in high density populations benefit from early emergence in the intense scramble competition for mates. In low density populations, however, scramble competition is reduced or absent, and theoretical models predict that protandry will be less favoured. This raises the question of how males behave in heterogeneous landscapes characterized by high density core populations in a low density continuum. I hypothesized that disadvantaged late emerging males in a core population would disperse to the continuum to find mates. I tested this idea using the protandrous, monandrous, pierid butterfly *Anthocharis cardamines* (the orange-tip) in a core population in Cheshire, northwest England. Over a six-year period, predicted male fitness (the number of matings a male can expect during his residence time, determined by the daily ratio of virgin females to competing males) consistently declined to <1 in late season. This decline affected a large proportion ($\sim 44\%$) of males in the population and was strongly associated with decreased male recapture-rates, which I attribute to dispersal to the surrounding continuum. In contrast, reanalysis of mark-release-recapture data from an isolated population in Durham, northeast England, showed that in the absence of a continuum very few males ($\sim 3\%$) emerged when fitness declined to <1 in late season. Hence the existence of a low density continuum may lead to the evolution of plastic dispersal behaviour in high density core populations, maintaining late emerging males which would otherwise be eliminated by selection. This has important theoretical consequences, since a truncated male emergence curve is a key prediction in game theoretic models of emergence timing which has so far received limited support. These results have implications for conservation, since plastic dispersal behaviour in response to imperfect emergence timing in core (source) populations could help to maintain sink populations in heterogeneous landscapes which would otherwise be driven to extinction by low mate encounter-rates (Allee effects).*

[This Chapter formed the basis for the paper published by Davies and Saccheri, 2015]

Introduction

In the previous Chapter, it was shown that male emergence timing in *A. cardamines* is affected by host-plant utilization, and it was pointed out that in dense core populations where both chief host-plants occur (such as Dibbinsdale), late emerging males which have utilized *A. petiolata* will likely be disadvantaged relative to early emerging ones which have utilized *C. pratensis*. In this Chapter, I discuss the general theory of protandry and analyze whether male emergence timing affects male fitness

in the Dibbinsdale population, and if so how disadvantaged males might respond to this situation.

Protandry, in its broadest sense, denotes the input of males before females into breeding areas (Morbey & Ydenberg, 2001). In insects, where mating usually takes place close to the eclosion (emergence) site, protandry refers more specifically to the prior emergence of males (Wiklund & Fagerstrom, 1977). This widespread phenomenon has repeatedly been analyzed in terms of direct selection pressures acting on emergence timing (Wiklund & Fagerstrom, 1977; Botterweg, 1982; Fagerstrom & Wiklund, 1982; Bulmer, 1983; Iwasa et al., 1983; Parker & Courtney, 1983; Zonneveld & Metz, 1991, Iwasa & Haccou, 1994). Although incidental explanations based on independent selection for correlated traits are also possible (Morbey & Ydenberg, 2001), these are considered unlikely in insects (e.g. Wiklund & Solbreck, 1982; Wiklund, Wickman & Nylin, 1992; Holzapfel & Bradshaw, 2002; but see Matsuura, 2006).

The key requirements for the evolution of protandry in insects are that generations are discrete, so that prior male emergence is possible (Singer, 1982), and that females mate only once (monandry), so that late emergence is costly to males (Wiklund & Fagerstrom, 1977). The earliest mathematical attempts to explain protandry were optimality models based on the assumption that maximum reproductive success coincides with peak emergence in males (Wiklund & Fagerstrom, 1977) or with peak male availability in females (Fagerstrom & Wiklund, 1982). Thus, only the average date of emergence was taken to be under selective control; the variance around this date was considered to be caused by environmental noise (Fig. 3.1A). Reproductive success in males was measured by the number of matings expected over their lifetime (based on the relative numbers of virgin females to male competitors through the season) or in females by the rapidity with which they were mated (based on the number of males in the population at the time of their eclosion). These models worked well in so far as an earlier emergence date was predicted for males, whether males were considered to be selected in response to the female emergence curve (Wiklund & Fagerstrom, 1977) or vice versa (Fagerstrom & Wiklund, 1982). An alternative model, based on the equilibration of the male emergence curve at the point where directional selection on its mean ceases, was also successful in predicting protandry (Bulmer, 1983).

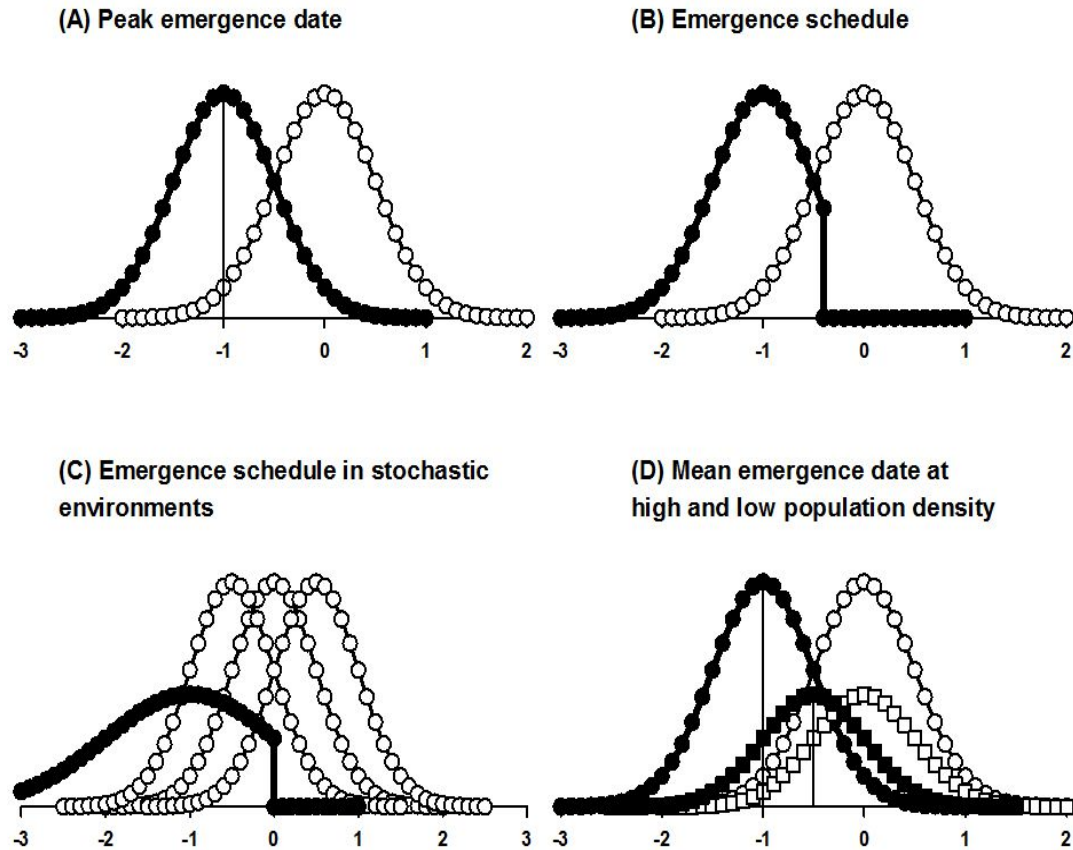


Figure 3.1 Comparison of the key features of different protandry models. In all cases, selection modifies male emergence date (filled symbols) in response to the female emergence curve (open symbols). Abscissa = emergence date (arbitrary units); ordinate = number emerging. (A) Protandry results from selection on peak male emergence date; (B) frequency-dependent selection modifies the shape, as well as the mean, of the male emergence curve, which is predicted to be truncated; (C) a bet-hedging strategy increases the variance of the male emergence curve in response to stochasticity in female emergence date (here represented by varying position of female emergence curve); (D) mate encounter rate modifies degree of protandry, which is more pronounced in high (circles) than in low (squares) density populations (ordinate = number *per unit area* (density) emerging; this model reverts to the assumption that only the mean emergence date is modified by selection).

The validity of the assumption that only the average emergence date is modified by selection was challenged in several game-theoretic models in which it is replaced by the hypothesis that specimens emerging at any point in the season gain equal fitness (Bulmer, 1983; Iwasa et al., 1983; Parker & Courtney, 1983). This situation is assumed to evolve in response to frequency-dependent selection: males emerging in peak season will encounter the highest number of females but will also be in competition against the highest number of males, whereas those emerging at other times will encounter fewer females but will also have fewer competitors. In order to balance the fitness of specimens emerging at any point in the season exactly, the

male emergence curve must take a specific form in relation to the female emergence curve. Hence, both the peak and the shape of the male emergence curve are envisioned as responding to selection, which pushes the emergence schedule towards an ideal free distribution or evolutionarily stable strategy (ESS). If female emergence timing is treated as an independent variable, then the corresponding ESS male emergence schedule can be solved for it either analytically or by simulation. (Game theorists have so far paid little attention to how the female emergence schedule might respond to male emergence timing (but see Zonneveld & Metz, 1991)).

The results of the early game-theoretic models were mixed. A key prediction of the analytic models was that the male emergence curve should be truncated (Bulmer, 1983; Iwasa et al., 1983), with no males emerging after a specific date in the season (Fig. 3.1B); this was not observed in careful studies of the checkerspot butterfly *Euphydryas editha* (Iwasa et al., 1983; Baughman, Murphy & Ehrlich, 1988). Iwasa and Haccou (1994) suspected that deterministic game-theoretic models, which neglected stochastic noise, relied too heavily on the implicit assumption that organisms possess extremely accurate emergence cues which would enable them to perfectly compensate for the effects of a fluctuating environment. This led them to examine the impact of stochastic effects on the male emergence curve in relation to a bet-hedging strategy, in which specimens of the same genotype emerge at different times to maximize their average logarithmic reproductive success. They found that, in the absence of an accurate cue, such a strategy increased the variance in the emergence curve compared with that predicted by a deterministic model (Fig. 3.1C); in the presence of a perfect cue, the emergence schedule is identical to the one predicted by the deterministic model. This work was important in stressing the relevance of environmental noise and emergence cues in the evolution of protandry (Sawada et al., 1997), but the problems relating to the prediction of a truncated emergence remained.

The simulation model of Parker and Courtney (1983) fared rather better when applied to the orange-tip butterfly, *Anthocharis cardamines*. In a highly localized population in Durham in northeast England, the observed distribution of male eclosion times closely matched the predicted ESS distribution (whether calculated on the assumption that females were (partly) polyandrous or monandrous). Interestingly, the male emergence curve for this population did terminate abruptly (neglecting the

contribution of a small number of late emerging specimens, and within the limits set by the summation of emergences over successive 4-day periods). These results indicate that strong selection is capable of effectively modifying male emergence timing in this species.

A key assumption in all the protandry models discussed so far is that females are mated on the day of eclosion, so the presence curve for virgins is identical to the emergence curve. In low density populations, where mate encounter rates are low, this is unlikely to be true. Theoretical evidence that protandry should evolve in response to population density was provided by Zonneveld and Metz (1991). Their analysis reverted to the assumption that only mean emergence time is under selective control, and they used a ‘law of mass action’ (density-dependence) to model male-female encounter rates. They found that as encounter rates approach zero, the evolutionarily stable degree of protandry also approaches zero; hence, protandry should be diminished or absent in low density populations (Fig. 3.1D). These results are in agreement with those of the earlier simulation model of Botterweg (1982) for the pine looper moth *Bupalus piniarius*, which showed that the expected degree of protandry decreases when both flight activity (males) and moth density (both sexes) are reduced to very low levels, i.e. when mate encounter rates are minimized.

In heterogeneous landscapes, population density will vary spatially, so protandry will be more favoured in some areas than in others. Specifically, protandry should be strongly selected in high density core habitats; if these are isolated or nearly so, and if selection is frequency-dependent, late emerging males should be eliminated, since a truncated emergence curve is a robust prediction of the analytic game-theoretic models (Iwasa & Haccou, 1994). If, however, the core habitats are not isolated, but connected to low density areas in which protandry is less favoured, late emerging males could increase their fitness (i.e. the number of matings they can expect) by emigrating to them (Fig. 3.2).

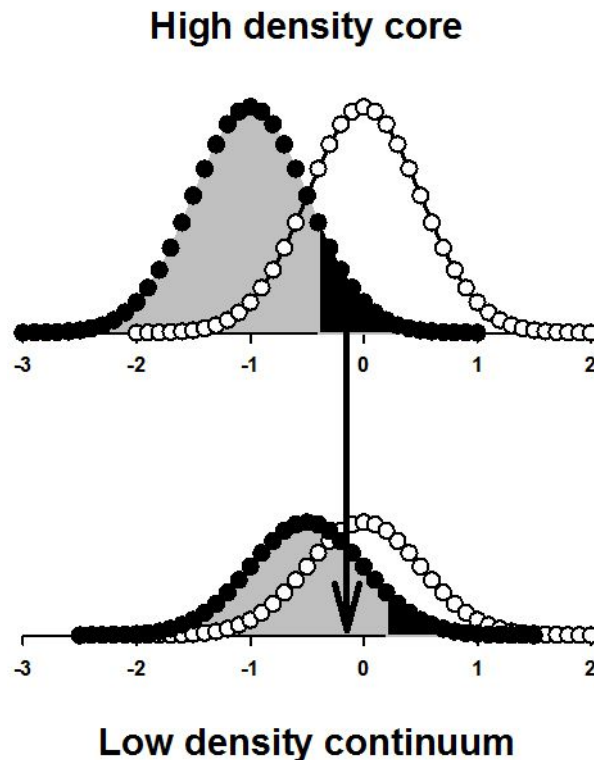


Figure 3.2 Schematic representation of hypothesized male behaviour in a high density core population immersed in a low density continuum. Models B and D in Fig. 3.1 predict that a truncated male emergence curve should evolve in response to frequency-dependent selection and that protandry should be more pronounced in high density populations. In heterogeneous landscapes late emerging core males could therefore improve their fitness by emigrating to low density areas; selection for such behaviour could prevent the evolution of a truncated emergence curve. Filled and open circles show male and female emergence curves; grey and black shading represent adaptive and maladaptive male emergence timing in isolated populations, with the predicted truncation date lying at the boundary between them; the arrow shows the fitness benefit gained by late emerging core males in dispersing to the continuum; abscissa - arbitrary emergence date; ordinate - relative density emerging.

The *A. cardamines* population studied by Parker and Courtney (1983) was nearly isolated, so the close correspondence of the male emergence curve to the predicted ESS is in line with the above hypothesis; late emergent males are unable to recover fitness by emigrating to a low density area and so are eliminated. On the other hand, the core population in Dibbinsdale is immersed in a much wider area over which the butterfly is continuously but thinly distributed (here termed a 'continuum') (Fig. 3.3). Hence the Dibbinsdale population provides an excellent study system to investigate the following key questions:

1. Is there a consistent decline in predicted male fitness through the flight season, indicating that if the core habitat were isolated, late emerging males would be eliminated (as in the population studied by Parker and Courtney)?
2. Do disadvantaged late season core males emigrate to the continuum?



Figure 3.3 *A. cardamines* pupa (shuttle shaped object, centre) on *A. petiolata* host-plant growing on a road verge outside Dibbinsdale (Poulton Hall Road, right), demonstrating that the butterfly breeds at low density in the 'continuum' surrounding the Reserve. It is very unusual for larvae of *A. cardamines* to pupate on the host-plant like this. Photographed by James Davies on 27 June 2007.

Methods

POPAN estimation of male and female emergence schedules

Mark-Release-Recapture (MRR) was undertaken (as described in Chapter 2) throughout the butterfly flight period for six study seasons (2005-2010). The daily 'input' (emergence and immigration) of males and females into the Dibbinsdale population in each year of the study period was estimated using the POPAN formulation in program MARK (Cooch & White, 2014). The usual approach to analyzing MRR data with MARK is to start with a general model and then modify it through parameter reduction. This results in a set of candidate models from which hypotheses relating to the behaviour of the marked animals can be tested. Since the number of sampling occasions in my data-sets were large (25-46 days), the most general time-dependent models which could have been constructed would have been extremely unwieldy and of very limited utility due to the sparseness of encounters outside peak season. Moreover, accurate information on the behaviour of the butterflies had already been obtained from analyses of residence plots (Chapter 2). Models were therefore constructed directly on the basis of this information (after

confirming there were no inherent problems with the MRR data by assessing goodness-of-fit with the *Release* tool in MARK). Different approaches were used for males and females.

Male behaviour is influenced by both size and time of appearance. Individuals were therefore assigned to separate attribute groups on the basis of wing-length and intra-seasonal period of first capture. Each group was assigned a separate constant survival probability (residence-rate) and the encounter probability was set to the same constant in all groups (as sampling effort was uniform). The total number of new entrants into the population on each day of the season was obtained by summing the contributions from each group.

There is no conclusive evidence that size or time of appearance affects the behaviour of females. However, there was a problem with unequal catchabilities among different sub-sites of the Reserve in 2005 and 2006, when females were more commonly encountered in some sub-sites than in others. These were accordingly assigned to separate attribute groups with distinct encounter probabilities; the survival probability was set to the same constant in the two groups. In 2007-2010, the problem of unequal catchabilities did not arise, so there was just one group with a single survival and encounter probability.

The daily input curves are difficult to interpret visually. I therefore present simplified curves in which the input of males and females into the population in successive 4-d periods have been combined. In the male fitness models, however, daily inputs were used except for the reanalysis of the data from Parker and Courtney (1983), in which I used the 4-d input intervals and 4-d residence-rate given by the authors.

Male fitness model

For the calculation of predicted male fitness, I exclude the possibility of increased emigration in late season, since this is hypothesized to evolve in *response* to the situation I am trying to uncover. I therefore assume that male residence time is constant (equivalent to assuming a constant daily residence-rate - see below), and use the fitness model of Parker and Courtney (1983) to test the null hypothesis that observed differences in emergence timing do not affect the number of matings males obtain in the Dibbinsdale population. The Parker-Courtney model assumes that on

any day of the flight season the number of matings a male achieves is directly proportional to the number of newly emerging (virginal) females and inversely proportional to the number of male competitors. Hence, females are assumed to be mated on the day of their emergence, and male fitness is a direct consequence of scramble competition for mates. The number of males present on day t (m_t) is calculated from

$$m_t = \sum_{n=1}^{n=t} M_n s^{t-n} \quad (3.1)$$

where M_n is the number of males entering the population on day n (from POPAN), and s is their daily residence-rate (derived from whole-season residence plots - see below); the factor s^{t-n} corrects for the loss of specimens between days n and t . The fitness (λ) of males entering the population on day j is therefore

$$\lambda_j = \sum_{n=j}^{n=T} \frac{F_n}{m_n + 1} s^{n-j} \quad (3.2)$$

where F_n is the number of females entering the population on day n (from POPAN) and T is the termination date of the season; the factor s^{n-j} is the probability that a specimen entering the population on day j is still present $n - j$ days later. (I have added 1 specimen to the denominator of the Parker-Courtney model to prevent it from tending to zero at times when the population is very sparse). For each year in the study period, the total number of females entering the population was adjusted to equal the number of males, since in captivity the sex-ratio is equal (an equal sex-ratio in the field is generally supported by the approximate estimations obtained from POPAN). All calculations were executed on a spreadsheet in *Excel*.

The average fitness (λ_{av}) of males entering the population during a specific intra-seasonal period was obtained from

$$\lambda_{av} = \frac{\sum_{j=y}^{j=z} M_j \sum_{n=j}^{n=T} \frac{F_n}{m_n} s^{n-j}}{\sum_{j=y}^{j=z} M_j} \quad (3.3)$$

where the intra-seasonal period runs from days $j = y$ to $j = z$. I have here dropped the addition of 1 to the denominator of the fitness term since $m_n \rightarrow 0$ implies $M_j s^{n-j} \rightarrow 0$, and so the term vanishes from the summation.

There are three caveats which must be taken account of when considering the applicability of this model.

1. Not all females entering the population will be virginal, due to the immigration of mated individuals from outside the study area. However, provided the immigrants do not alter the *pattern* of the true emergence curve (the temporal variation in the relative number of virgin females entering the population) this effect may be neglected. Since the Dibbinsdale population is highly concentrated (Fig. 1.5), it is unlikely that the immigrant flux will be large enough to swamp the true emergence curve (it is certainly insufficient to obscure differences in wing-length distribution between sub-sites). The same considerations apply to the male emergence curve. Hereafter, I refer to all input curves as emergence curves, neglecting the contribution of immigrants.

2. Females have been assumed to be monandrous, so that only virgins are available for mating. This is largely correct for *A. cardamines*, although polyandrous females are known (Courtney, 1980; Wiklund & Forsberg, 1991). Again, these latter are unlikely to alter the broad pattern in the temporal availability of receptive females.

3. The model implicitly assumes a homogeneous environment in which the emergence curves are uninfluenced by micro-climatic variation. Ideally, I would have liked to study separate eclosion patterns in different sub-sites within the Reserve, but the scarcity of female captures was prohibitive. However, serious problems would only arise if emergence timing was highly asynchronous between sub-sites, which is unlikely. Furthermore, I restrict my key conclusions to inferences drawn from large changes in average male fitness between lengthy intra-seasonal periods each year; such coarse-scale effects should be unaffected by asynchrony between sub-sites, or by daily sampling artefacts, as is evidenced by their repeatability between years.

Reanalysis of data from Parker and Courtney (1983)

I compare my results, obtained for a high density core population located within a low density continuum, with those obtained by Parker and Courtney (1983) for an almost completely isolated population of *A. cardamines* in Durham in 1977. For the male emergence curve, I used the data given explicitly by Courtney (1980); for the female emergence curve, I measured the input from Fig. 2 of Parker and Courtney (1983). Parker and Courtney did not exclude poor weather days, and their emergence curves are summed over 4-d intervals. Accordingly, these curves were used in conjunction with the 4-d residence-rate (given by Parker & Courtney, 1983) to estimate male fitness at 4-d intervals from equation 3.2.

Intra-seasonal changes in male behaviour

The prediction that late season males emigrate from the core population was tested indirectly. I first develop a novel method for identifying two co-occurring behavioural phenotypes in a wild insect population, when one of the phenotypes has a very short residence time. These phenotypes must differ in either their death or emigration-rates. I then apply this method to my data and argue that the co-occurrence of two phenotypes in late season probably represents a dispersal polymorphism.

Theory

The daily probability that an animal will be retained in a population is described by its *residence-rate* (Watt et al., 1977), which is influenced by both survival and movement; the higher the residence-rate, the longer the *residence time*. In insect populations, the residence-rate is usually constant with age (at least up to the time of senescence). In this case, the number of individuals remaining against time elapsed since first capture will decline exponentially; logarithmic transformation will then yield a straight line *residence plot* (termed a recapture-duration decay-plot by Watt et al., 1977) whose gradient is determined by the residence-rate. Hence the residence-rate can be obtained from the gradient of a residence plot for MRR data. This will represent the average phenotype in a population, determined by the average vulnerability to death and the average propensity to emigrate. If the residence-rate changes, then the average death-rate and/or emigration-rate will have changed.

Strictly speaking, the residence-rate obtained from a residence plot only applies to specimens recaptured at least once, since it is derived from the time elapsed between first and last capture. Hence, specimens disappearing from the population very rapidly after first capture do not influence the calculated residence-rate. This leaves open the possibility that a distinct phenotype with a very high death/emigration rate might exist in the population which does not impact on the residence plot, since so few recaptures of it are actually made.

If the fraction of specimens recaptured declines through the season, there are two possibilities (Fig. 3.4).

(A) The residence-rate of the average phenotype has decreased, due to an increase in either the death or emigration-rate across the entire population. In this case, fewer recaptures are made due to the shorter residence time of the average phenotype.

(B) A second phenotype with very high death/emigration rate has appeared in the population alongside the average phenotype. In this case, fewer recaptures are made due to the rapid disappearance of the new phenotype.

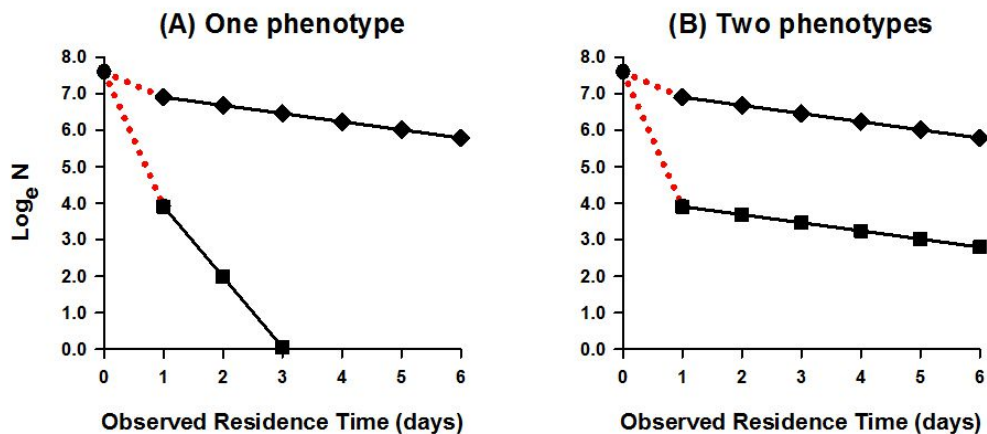


Figure 3.4 Hypothetical residence plots showing alternative explanations for a low number of recaptures. The zero day data point is the number of specimens initially captured and released, and the day 1 data point is the number of specimens recaptured at least once; red dotted lines indicate the number of specimens never recaptured. If the residence-rate of the average phenotype is high (gradient of regression line shallow), specimens will remain in the population for a long time and a high number of recaptures is predicted (top line, diamonds). If the observed number of recaptures are low, there are two possibilities. (A) The residence-rate of the average phenotype has declined (gradient of regression line steep), so specimens rapidly exit the population and avoid recapture (bottom line, squares). (B) A second phenotype has appeared in the population with a very high death/emigration rate; these avoid recapture and do not impact on the calculated residence-rate of the average phenotype (bottom line, squares), but their presence can be deduced if the number of recaptures is significantly lower than would be predicted with the residence-rate of the average phenotype.

To distinguish these possibilities, the residence-rate specific to the period when recaptures are low should be calculated, which allows estimation of the corresponding *predicted* number of recaptures (see below and Appendix 4). If this does not differ significantly from the observed number, then the death/emigration rate of the average phenotype in the population is sufficient to explain the low number of observed recaptures. If, however, the observed number of recaptures is significantly lower than the expected number, then the average phenotype is accompanied by a second phenotype with a very high death or emigration-rate.

Method

Residence and recapture plots (from whose gradient the encounter-rate can be derived, see Appendix 4) were prepared from the MRR data for each year of the study period; best-fit lines were fitted using least-squares regression in *Excel*. The fraction of specimens predicted to be recaptured at least once (F) in each year was calculated from the formula

$$F = e^{m/2} \frac{g}{g + m} \quad (3.4)$$

where m = gradient of best-fit line of residence plot and g = gradient of best-fit line of recapture plot. The derivation of this equation is given in Appendix 4.

The number of specimens predicted (or expected) to be recaptured at least once (E_R) for any intra-seasonal period was calculated as $N.F$, where N is the number of specimens initially caught and released during that period. The standardized residual (ΔR) of the observed number of recaptures (O_R) from the expected number was calculated as

$$\Delta R = \frac{O_R - E_R}{\sqrt{E_R}} \quad (3.5)$$

If ΔR is positive/negative then more/less specimens were recaptured than predicted. This allows inter- and intra-seasonal changes in the proportion recaptured to be interpreted in terms of standardized departures from expectation.

Results

The male and female eclosion curves were not Gaussian; the pattern of emergences was usually complex and differed among seasons (Fig. 3.5). The male emergence curve was not truncated. The fitness curves (Fig. 3.5) derived from the whole-season residence-rates (Table 3.1) and emergence curves, under the assumption that all males have equal residence times, show that the number of matings a male can expect in the Dibbinsdale population varies with his eclosion time: males emerging early in the season are generally predicted to have higher fitness (≥ 1) than those emerging later (< 1). (In some years, males emerging 'too early' are also predicted to have low fitness, but this effect is intermittent). Hence, the null hypothesis that emergence timing does not affect male fitness, assuming all residence times are equal, can be rejected.

Table 3.1 Summary of data obtained from residence and recapture plots in each year of the study period. The slope of the best-fit line for the residence plot (m) was used to calculate the daily residence-rate (s) as $\exp(m)$ and the average residence time (RT) as $-1/m$. The fraction of specimens predicted to be recaptured at least once (F) was calculated from equation 3.4.

Year	m	S.E.	g	S.E.	s	RT	F
2005	-0.169	0.006	-0.501	0.019	0.845	5.92	0.687
2006	-0.374	0.020	-0.758	0.011	0.688	2.67	0.555
2007	-0.289	0.014	-0.813	0.035	0.749	3.46	0.639
2008	-0.152	0.008	-0.851	0.055	0.859	6.58	0.786
2009	-0.162	0.006	-0.285	0.010	0.850	6.16	0.588
2010	-0.207	0.009	-0.654	0.040	0.813	4.83	0.685

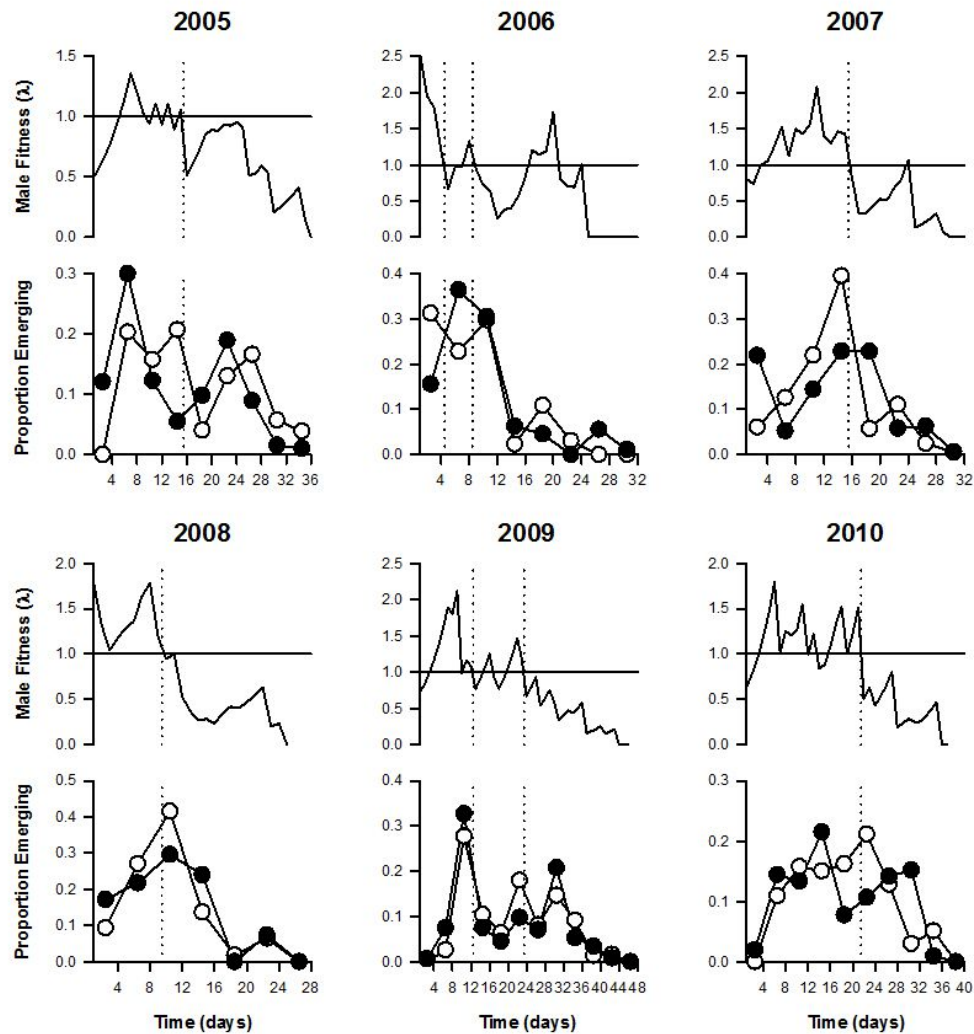


Figure 3.5 Predicted male fitness (upper graphs) in relation to male and female emergence curves (lower graphs) in the Dibbinsdale population for each year in the study period. The expected fitness (number of matings, λ) for males emerging on each day of the season was calculated from equation 3.2; a fitness of 1 is marked by the horizontal solid line. Male (filled circles) and female (open circles) daily emergences were estimated with POPAN and are summed over 4-day periods. Vertical dotted lines show the sub-division of each year into separate intra-seasonal periods based on long-term changes in predicted fitness (usually to a value <1 ; the peak in late season 2006 has been neglected since it only corresponds to a small number of specimens; high fitness periods in early season 2006 and 2009 have also been distinguished).

When the emergence curve is partitioned into different intra-seasonal periods on the basis of long-term changes in the accompanying fitness curves (Fig. 3.5), it is found that a high percentage (44% on average) of males consistently emerge after their predicted fitness declines to an unsustainable level (<1) in late season (Table 3.2). This contrasts strongly with the isolated Durham population studied by Parker and Courtney (1983), in which the late season decline in fitness to a value <1 coincides

with a sharp decline in male emergence frequency (Fig. 3.6) such that only 3% of the population emerged after this date (Table 3.2).

Table 3.2 Mean late season male fitness ($\bar{\lambda}$, from equation 3.3) against percentage emerging (from POPAN) in the Cheshire (Dibbinsdale) and Durham *A. cardamines* populations.

Population	Year	$\bar{\lambda}$	% Emerging
Cheshire	2005	0.83	40
	2006	0.68	48
	2007	0.45	36
	2008	0.61	61
	2009	0.67	37
	2010	0.46	41
Durham	1977	0.80	3

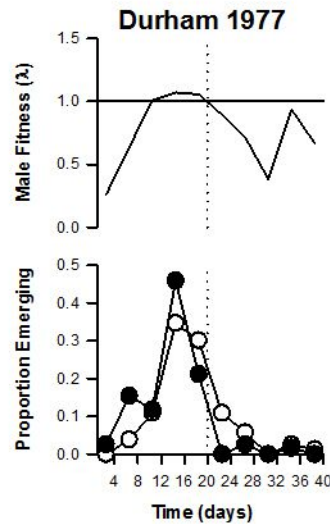


Figure 3.6 Male (filled circles) and female (open circles) emergence curves and estimated male fitness (both summed over 4-day periods) for an isolated population of *A. cardamines* in Durham in 1977 (reanalysed from data in Parker and Courtney, 1983). Note the sharp decline (truncation) in male emergence frequency after predicted fitness declines to <1 (vertical dotted line); in contrast, the female emergence curve is not truncated.

The presence of a high proportion of disadvantaged late emerging males in the Dibbinsdale population raises the question as to whether they remain within it (and hence whether the assumption that all males have equal residence times is valid). Males emerging in different intra-seasonal periods were recaptured at different rates. In all years, the fraction of males recaptured at least once declined through the season (Table 3.3). When the observed number of recaptures is compared with the expected number (on the hypothesis that all males belong to the same phenotype characterized by the whole-season residence-rates given in Table 3.1) it is found that in late season

the number of recaptures always falls significantly below expectation (results of χ^2 tests given in Table 3.3). In earlier intra-seasonal periods the observed number of recaptures was usually close to the expected number, except in 2005, when it was significantly below expectation, and in 2006 and 2009, when in early season it was significantly above expectation.

Table 3.3 Variation in male average fitness ($\bar{\lambda}$) and recapture frequency with intra-seasonal period (specified by long-term changes in daily fitness; see Fig. 3.5). N = number of males captured during the specified period, O_R = observed number of recaptures, E_R = expected number of recaptures ($= NF$, where F is taken from Table 3.1). The results of χ^2 tests on the significance of the departures from expectation are also shown.

Year	Period (d)	$\bar{\lambda}$	N	O_R	E_R	χ^2	P
2005	01 - 15	1.12	66	35	45.4	7.585	0.006
	16 - 36	0.83	33	10	22.7	22.696	<0.001
2006	01 - 04	2.23	20	18	11.1	9.630	0.002
	05 - 08	0.90	41	22	22.8	0.057	0.811
	09 - 32	0.68	43	10	23.9	18.121	<0.001
2007	01 - 15	1.32	82	50	52.4	0.294	0.588
	16 - 32	0.45	47	14	30.0	23.627	<0.001
2008	01 - 09	1.47	33	22	25.9	2.813	0.094
	10 - 25	0.61	39	12	30.7	53.180	<0.001
2009	01 - 12	1.24	52	40	30.6	7.081	0.008
	13 - 26	1.13	51	27	30.0	0.712	0.399
	27 - 46	0.67	37	13	21.7	8.521	0.004
2010	01 - 21	1.32	71	46	48.6	0.444	0.505
	22 - 36	0.46	38	17	26.0	9.909	0.002

Multiple regression analysis showed that predicted male fitness but not year had a significant effect on the standardized residuals of the observed minus expected number of recaptures (ΔR). When year was removed from the model, a simple linear regression confirmed that the relationship between ΔR and predicted male fitness was strongly positive and highly significant (Fig. 3.7). If lower than expected recapture rates (negative ΔR) result from faster dispersal, these data support the hypothesis that males emigrate from the study area at times when their future mating prospects are low.

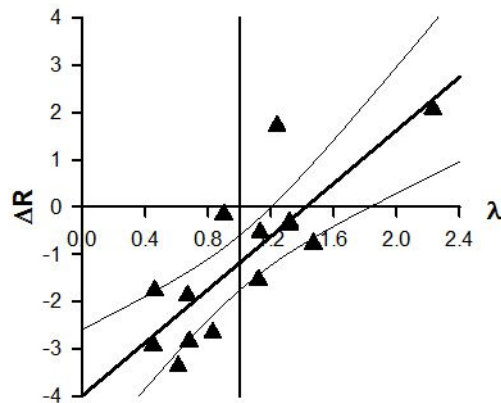


Figure 3.7 Regression (with 95% confidence band) of ΔR (standardized residuals from expected number of recaptures, from equation 3.5) on male fitness (average number of matings, $\bar{\lambda}$). The vertical line marks a fitness of 1.0. Regression equation: $\Delta R = 2.81 \bar{\lambda} - 3.99$, $R^2 = 0.67$, $p = 0.0004$. (Note that the two data points from early-season 2007 and early-season 2010 overlap (see Table 3.3); there are actually 14 data points contributing to the regression.)

In most seasons (2005-2009), reanalysis of late season data using the gradients of residence plots restricted to that period (e.g. 2009, Fig. 3.8) show that there were two co-occurring phenotypes in the population at that time. In those years, the late season deficit in the number of recaptures remained highly significant, even when the residence-rate of recaptured specimens decreased (Table 3.4). Therefore, even if the death/emigration rate of the average phenotype had changed in late season, it cannot wholly account for the low number of recaptures obtained during that period. Instead, this must be attributed to a very rapid loss of specimens from the study area shortly after first capture, and, by implication, shortly after emergence. While this might suggest that some butterflies undertake an escape flight, such behaviour has never been observed in the field, and it certainly does not occur in early season, or amongst recaptured specimens in late season. The excess specimens never recaptured must belong to a second phenotype characterized by a very high death or emigration-rate.

The only exception to this pattern occurred in 2010, when the low number of recaptures in late season can be wholly accounted for by a decreased residence-rate (Table 3.4); since late-season specimens had a shorter average residence time (2.1

days) than those emerging earlier (5.5 days), fewer were recaptured. In this case the average phenotype changed between early and late season, with the late season specimens exhibiting a higher death and/or emigration rate than the early season ones (Fig. 3.8).

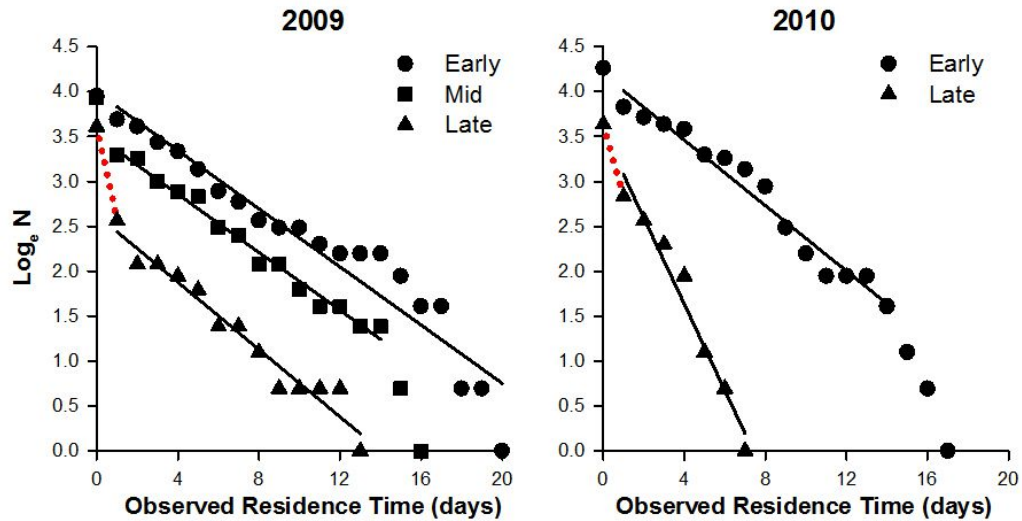


Figure 3.8 Intra-seasonal residence plots for 2009 and 2010. The residence-rate is related to the gradient (m) of the plots; the steeper the slope, the faster specimens depart (die/emigrate) from the population and the lower the residence-rate. In 2009, recaptured butterflies behaved uniformly through the season ($m \pm \text{SE} = -0.162 \pm 0.011$ for early (1-12 d) specimens; -0.162 ± 0.006 for mid (13-26 d) specimens; -0.187 ± 0.012 for late (27-46 d) specimens); therefore the low number of late season recaptures must be attributed to the appearance of a new phenotype which evaded recapture due to a high death/emigration rate (red dotted line). In 2010, recaptured late season butterflies exited the population more quickly than early season ones ($m \pm \text{SE} = -0.182 \pm 0.010$ for early (1-21 d) specimens; -0.480 ± 0.041 for late (22-36 d) specimens); this was sufficient to account for the low number of recaptures during that period (red dotted line). In this case, the behaviour of the average phenotype had changed through the season. Compare Figure 3.4.

Table 3.4 Reanalysis of late season recapture data using the gradients (m) of residence plots restricted to that period to recalculate the predicted fraction of recaptures (F). For 2005-2009, the observed number of recaptures (O_R) falls significantly below the expected number (E_R), even when the late season values of m are steeper than the whole season values (shown in Table 3.1). For 2010, the late season value of m removes the recapture deficit obtained with the whole season value (Table 3.1).

Year	m	S.E.	F	N	O_R	E_R	χ^2	P
2005	-0.163	0.017	0.695	33	10	22.9	23.919	<0.001
2006	-0.523	0.040	0.456	43	10	19.6	8.622	0.003
2007	-0.275	0.037	0.651	47	14	30.6	25.841	<0.001
2008	-0.246	0.021	0.686	39	12	26.8	25.906	<0.001
2009	-0.187	0.012	0.550	37	13	20.3	5.894	0.015
2010	-0.480	0.041	0.454	38	17	17.2	0.006	0.938

The co-occurring late season phenotypes in 2005-2009 could differ in their death or emigration rates. To try to distinguish between these possibilities, note that if the

rapid loss of a high number of late season males were due to death, it would be unaffected by spatial scale, since a dead individual can never be recaptured no matter how wide the area in which we search. Therefore, estimates of the recapture shortfall (i.e. the expected minus observed number of recaptures) should not be biased higher or lower when calculated on the scale of the sub-sites of first-capture (SSFC) compared with the whole-site (WS). On the other hand, if the losses were due to dispersal, some specimens might be recovered in the WS after they have left their SSFC, so the recapture shortfall is predicted to be smaller in the WS.

The recapture shortfall is always smaller when calculated on the scale of the WS (Table 3.5); the chances of this happening in 5 successive years, when the estimates are expected to vary at random (i.e. when there is an equal chance that the calculated shortfall will be higher or lower in the WS), is $0.5^5 = 0.03$. Over the 5 year period, about 20 specimens in the recapture shortfall for the SSFC were later recovered elsewhere in the WS. These losses were therefore due to dispersal rather than death. It is therefore likely that most of the remaining 70 specimens in the recapture shortfall had dispersed to the continuum (where they were unavailable for recapture).

Table 3.5 Late season recapture shortfall (expected minus observed number of recaptures) calculated from the gradients of the residence and recapture plots specific to sub-sites of first-capture (SSFC) and the whole-site (WS).

Year	SSFC	WS
2005	13.2	12.7
2006	16.4	13.9
2007	20.4	16.0
2008	21.5	18.7
2009	18.8	8.7
Total	90.3	70.0

Discussion

Interpretation of results

A simple model of male fitness in a core population of *A. cardamines* in northwest England shows that, under the assumption that all males have equal residence times, the number of matings a male can expect consistently declines to <1 in late season. Hence, the null hypothesis that (on the long average) males emerging at any time in the season can expect the same number of matings is falsified. The large proportion of males (~44%) emerging in late season requires explanation.

In five of the six years in the study period, there were two sharply contrasting male phenotypes in late season. The residence-rate of the first of these did not differ, or differed relatively little, from that of the early season butterflies. The second phenotype was specific to late season and had a very low residence-rate, entailing a very high death or emigration-rate. I think the latter possibility far more likely.

Since only one of the two co-existing phenotypes in late season exhibits a low residence-rate, it is not the case that the *whole* population suffers an increased death/emigration rate at this time. This constrains the range of plausible explanations invoking an increased death-rate. In particular, increased mortality caused by deterioration in the physical environment or increased predation-rate can be ruled out, since all butterflies would be affected. Instead, we require a cause of mortality that would impact heavily on some butterflies but not on others. If some late season butterflies emerge from the chrysalis diseased, this would partition the population into infected/uninfected phenotypes exhibiting sharply contrasting death-rates, as required. However, I think this very unlikely: the disease would have to be present in a high percentage of males year after year, nothing like it has ever been encountered when rearing butterflies from wild larvae, and no specimens caught in the field have ever shown any obvious signs of ill-health.

On the other hand, the occurrence of two sharply contrasting dispersal phenotypes in late season presents no such difficulties. Dispersal polymorphisms are common in nature. Moreover, the appearance of a migratory phenotype in our study population in late season is predicted by decreased mate availability at that time; lack of mates is a well established dispersal cue among many taxa. It is therefore likely that the newly appearing phenotype in late season is characterized by a very high emigration-rate; this interpretation is supported by the larger recapture shortfall obtained on a smaller spatial scale within the study site (Table 3.5), implying that some migratory specimens were recovered when the search area was widened.

In 2010, there was only one late season phenotype. This was characterized by a lower residence-rate than the early season butterflies. While an increase in the general death-rate in late season cannot be ruled out in this case, in view of the results obtained in all other years it seems likely that this too was at least partially an effect of rapid dispersal. Perhaps the migratory response was slower in this year,

allowing a significant number of dispersing specimens to be recaptured and hence affect the calculated residence-rate.

I suggest that the appearance of a migratory phenotype in late season is part of a 'stay-or-go' response, in which males respond to cues relating to the availability of females by either settling in the Reserve ('stay' response) or rapidly emigrating from it ('go' response). Hence, the assumption of equal residence times in the fitness model is violated. Therefore, the model does not capture the true fitness of late emerging males; rather it reveals what is *avoided* by the existence of the 'stay-or-go' response, providing an insight into its evolution and/or maintenance. Were the 'stay-or-go' response to fail, then late season males should be selected against, since their fitness would be consistently lower than early season ones.

The prediction that spatial variation in population density can impact on the evolution of emergence timing and dispersal in heterogeneous landscapes (Fig. 3.2) is therefore supported by the data presented in this Chapter. In particular, there is evidence that selection for a truncated male emergence curve has been weakened in a high density core population by the evolution of a 'stay-or-go' response, which enables disadvantaged late emerging males to recover fitness by emigrating to a low density continuum. In contrast, the male emergence curve is truncated (or nearly so) in the isolated population studied by Parker and Courtney (1983), where a 'stay-or-go' response would be ineffective.

Evolution of emergence timing and dispersal in heterogeneous landscapes

Late emerging core males which emigrate to the continuum will not necessarily achieve the same fitness as earlier emerging ones, whose reproductive success is predicted to be >1 . Instead, they behave in the same way as subordinates do in populations exhibiting source-sink structure (Pulliam, 1988), by moving from the core (source) habitat to a nearby (sink) area to increase their reproductive success above the level achievable in the core (they effectively "make the best of a bad job" (Maynard Smith, 1982)). Pulliam (1988) showed that such a strategy would be evolutionarily stable, and could result in the maintenance of sink populations which would otherwise go to extinction.

The number of matings obtained by late emerging core males which migrate to the continuum will depend on the trade-off between improved input timing and decreased mate encounter-rates. If the population in the continuum is too sparse, then they will not improve their fitness by emigrating to it, and so late emergence should be selected against, driving the male emergence curve as far as possible towards an ESS. This appears to have happened in the isolated Durham population studied by Parker and Courtney (1983). However, the attainment of an ESS will ultimately depend on how far the shape of an emergence curve can depart from Gaussian (Bulmer, 1983), and, in stochastic environments, on the accuracy of emergence cues (Iwasa & Haccou, 1994). My own data show that the emergence curve for *A. cardamines* can depart significantly from Gaussian, while Parker's and Courtney's data imply that male emergence cues can be very accurate.

If mate encounter-rates in the continuum are high enough, late emerging core males will improve their fitness by migrating to it, since protandry is less favoured in low density populations (Zonnerveld and Metz, 1991). The evolution of an ESS emergence schedule could then be prevented by selection for late season dispersal. Since the emigration of competitors will reduce mate competition in the core, the fitness of males which stay behind will also increase. Hence frequency-dependent selection probably explains the evolution of the 'stay-or-go' response observed in the Dibbinsdale population of *A. cardamines*. The long term maintenance of late emerging males in core populations will ultimately depend on the descendants of the 'go' individuals returning to them; although reproductively successful 'stay' males eclosing near the end of the season could perpetuate late emergence short term, one way loss of genes triggering the 'go' response would eventually lead to the intense level of scramble competition predicted to select against late eclosion.

In the absence of scramble competition in the low density continuum, male fitness should be maximized when virgin females are most abundant. While I have no information on the emergence schedule in the continuum, it is interesting that male fitness in the source tends to decline sharply around the time of peak female emergence (Fig. 3.5). If the female emergence schedule does not vary across the landscape, the onset of the 'stay-or-go' response in the source will be synchronized with peak female density in the continuum. This suggests that the appearance of the 'go' phenotype in the source is optimized with respect to the continuum emergence

schedule. If so, this would account for the near instantaneous emigration of the 'go' males to the continuum, and hence for the sharply contrasting behaviours of the 'stay' and 'go' phenotypes in the source.

The 'stay-or-go' response in the Dibbinsdale population of *A. cardamines* most likely results from a genotype-by-environment interaction, since it is strongly associated with predicted fitness (Fig. 3.7), suggesting that males are capable of adjusting their behaviour in response to environmental cues ("condition-dependent" or "informed" dispersal in recent literature (e.g. Clobert et al., 2009; Chaput-Bardy et al., 2010, Hovestadt, Mitesser & Poethke, 2014). In this connection, it is interesting that males were sometimes (2006 and 2009) recaptured in excess in high fitness windows in early season (Table 3.3), indicating that they were more philopatric than normal when the environment was particularly favourable. Nevertheless, genetic variability in the reaction norms underlying the response likely explains why, in late season, some males 'stay' while others 'go'. In general, if cues relating to mating opportunity are more readily available or reliable than emergence cues, this should lead to the evolution of plastic dispersal behaviour instead of an ESS emergence curve, as has apparently happened in Dibbinsdale.

In the last Chapter it was shown that male *A. cardamines* utilizing *C. pratensis* emerge earlier and are slower to disperse (since smaller) than those utilizing *A. petiolata*. This effect appears to be a weaker version of the 'stay-or-go' response uncovered here; interestingly, the 'stay-or-go' response itself does not appear to be affected by wing-length, so host mediated differences in phenology/dispersal appear to be independent of it (Appendix 5). Nevertheless, host mediated differences in phenology will increase the variance in emergence timing and hence contribute to the variance in male fitness necessary to drive the evolution of the 'stay-or-go' response (see below). Therefore, the disadvantage associated with late male emergence consequent upon *A. petiolata* utilization in dense core populations will be decreased or overturned by the 'stay-or-go' response; in particular, their larger size should enable those which switch to the 'go' response to locate widely scattered females more efficiently in the continuum due to their faster dispersal.

Implications for conservation: maintenance of sink populations

A sink population is one which cannot persist without immigration, since the average reproductive success within it is <1 (Pulliam, 1988). The emigration of disadvantaged late emerging male orange-tips from the core population in Dibbinsdale resembles the behaviour of subordinates in a source (see above). If the continuum is a sink, the influx of subordinate males could play an important role in its persistence by boosting the number of females emerging there which are mated. In Appendix 6, I show how variance in male fitness (σ^2), assumed to be generated by imperfect emergence timing, in a source population of size n is related to the size (n_{sk}) of the population which can be maintained in a sink, on the assumption that disadvantaged males emerging in the source emigrate to the sink. This leads to the following equation:

$$\Phi\left(\frac{\lambda g - 1}{\sigma}\right) = \frac{n_{sk}(\alpha - \lambda g)}{n\lambda g} \quad (3.6)$$

where λg is the average number of matings males can expect in the sink, α is the proportion of sink females mated, and Φ is the cumulative probability function of the normal distribution, which in this case gives the proportion of specimens emerging in the source with a lower predicted fitness than they would (on average) achieve in the sink, i.e. with fitness $< \lambda g$. The size of the sink population can be obtained from

$$n_{sk} = \frac{n\left(1 - \frac{1}{\lambda s}\right)\lambda g}{\alpha - \lambda g} \quad (3.7)$$

where λs is the average number of matings obtained by 'stay' males in the source. These equations show how the variance in predicted male fitness in the source can be maintained by the existence of a sink population; or, conversely, how the maintenance of the sink population may be dependent on this variance.

It is instructive to quantify the relationship between precision in emergence timing and the potential size of a sink population in the Dibbinsdale area, where the size of the source population is $n \approx 300$ (POPAN estimate). For simplicity, I assume all sink females are mated ($\alpha = 1$). The average predicted fitness of source males emerging in early/mid season is 1.33 (Table 3.3); since the model upon which this is based

neglects the emigration of 'go' specimens, the fitness of the 'stay' ones will be underestimated, so I set $\lambda_s = 1.40$. The average predicted fitness in the source in late season is 0.62 (Table 3.3); to make emigration to the sink viable, I set $\lambda_g = 0.75$. With these values, $n_{sk} \approx 260$ (equation 3.7) and $\Phi = 0.29$ (equation 3.6), so $\sigma^2 = 0.20$ (from tables). Therefore, a sink population of 260 can sustain imprecise emergence timing in the source which translates into a fitness variance of 0.20; conversely, if $\sigma^2 = 0.20$ in the source, 29% of males will emigrate to the sink, where they will help to maintain a stable population of 260.

This approximation demonstrates the extent to which landscape structure could impact on the evolution of emergence timing, and so offers an explanation as to why the predictions of protandry theory, based on the implicit assumption of a homogeneous landscape, may fail. It also indicates that male behaviour could be very important for the maintenance of sink populations. In the original model of Pulliam (1988), sink populations are maintained by successive (partial) repopulations by subordinates emigrating from sources; in the absence of these repopulations, the low reproductive success (<1) of specimens in the sink would lead to their eventual extinction there. While this process (which in insects would depend upon the movement of egg-laying source females) may contribute to the maintenance of *A. cardamines* sink populations, the model developed here indicates that an alternative mechanism may be equally important. Specifically, it is shown that Allee effects (decreased population growth with decreased population density due to reduced mate encounter rates) in sink populations can be prevented by the arrival of subordinate males (determined by emergence timing) from source populations. In this case, the sink population is not rescued by the repeated input of subordinate source females, but rather maintained by the redistribution of subordinate source males. The implications for conservation are clear: even if females appear to be adequately adapted to find widely scattered oviposition sites in a sink, their reproductive success may depend on males arriving from a source; if the source population goes to extinction, then so will the population in the sink.

The 'stay-or-go' response and geographic selection mosaics

Trait remixing is an important process shaping geographic selection mosaics (Thompson, 2005). The 'stay-or-go' response in the Dibbinsdale population will act

to redistribute traits across the continuum and ultimately into other core populations. This will oppose genetic differentiation among demes, unless the selection pressures acting within them are very strong. Instead, moderate dispersal rates should select for phenotypic plasticity (Scheiner and Holt, 2012; Scheiner, Barfield and Holt, 2012) to accommodate changing selection pressures across the landscape (assuming they are associated with reliable cues). It is not known how widespread the 'stay-or-go' response is, but in the Durham area accurate male emergence timing suggests that it may be absent (since it is hypothesized to evolve in response to inaccurate emergence timing, and to require a continuum, it is predicted to be absent in Durham, but this must be confirmed experimentally). The implication is that the genetic determination of selection mosaics may vary across the British mainland, being more dependent on phenotypic plasticity in some areas and on genetic differentiation in others.

It is instructive to consider the positive feedback mechanisms underlying the evolution of a plastic response to host use and the 'stay-or-go' response in the Dibbinsdale area. The earlier emergence of males utilizing *C. pratensis* will disadvantage those utilizing *A. petiolata* in populations where both host-plants are present. This likely assisted in the evolution of the 'stay-or-go' response, which redistributes disadvantaged late emerging males across the landscape. In turn, this will contribute to the maintenance of low density sink areas (by opposing Allee effects) and increase gene flow between source populations. Hence the plastic response to host use will be maintained; it will not be genetically canalized since both host-plants are regularly encountered by the descendants of dispersing individuals across the landscape. In the absence of such positive feedback mechanisms, the outcome of the coevolutionary interaction between the butterfly and its host-plants may be very different; in particular, the smaller size of the imagines in Irish populations, where the chief host-plant is *C. pratensis*, may be genetically determined (Majerus, 1979), suggesting that the plastic response has been canalized in that region.

Chapter 4

Impact of parasitism by *Phryxe vulgaris* on life-history evolution in *Anthocharis cardamines*

Abstract

The maintenance of small size remains an unsolved problem in insect life-history evolution. In females, exponential growth coupled with the size-fecundity relationship predict that a short extension in larval life will significantly increase fitness. One way to resolve this paradox is to invoke positive size-dependent predation, so that extended larval life is costly. The orange-tip butterfly, Anthocharis cardamines, is heavily parasitized by the tachinid fly Phryxe vulgaris in late larval life. I develop a mathematical model (accurately parameterized from field data) in which parasitoid attack rates are a power function of larval length. High attack rates are then sufficient to overturn strong directional selection for increased body-size, but fall short of maintaining strong stabilizing selection for an optimal wing-length. When attack rates are size-independent, high levels of parasitism weaken, but do not overturn, the fecundity benefit of large size; in this case, parasitism would have to act in concert with other factors (e.g. time constraints) to constrain body-size evolution. The duration of the final instar resting phase, which larvae usually spend on the host-plant, strongly affects larval survival when attack rates are high; an alternative trait, whereby larvae leave the host-plant immediately at growth termination, is probably an evolutionary response to this. Independently of visual conspicuousness, larvae vary in susceptibility to parasitism on different host-plants due to different growth trajectories. This may explain the strong oviposition preference of female butterflies for the host-plant with the lowest predicted parasitism rate.

Introduction

In this Chapter, I turn my attention to the coevolutionary interaction between *A. cardamines* and the tachinid fly *Phryxe vulgaris*, which parasitizes its larvae. Specifically, I investigate various mechanisms by which high levels of parasitism could impact the life-history evolution of *A. cardamines*.

Life-history evolution is governed by the trade-offs arising between traits associated with reproductive success (Roff 2002). In seeking an evolutionary explanation for an organisms' life-history strategy, dynamic optimality models incorporating the age schedule of reproduction and death are varied with respect to the traits considered to be most important. The model which maximizes reproductive success when integrated over the organisms' life-span predicts the fittest combination of trait values, which can then be compared with observed values (Roff 1986; Hochberg et al. 1992; Abrams and Rowe 1996; Peckarsky et al. 2001; Roff et al. 2005; Berger et

al. 2006; Gotthard et al. 2007; Relyea 2007; Rimmel et al. 2011). Such models have been very successful in predicting organisms' life-history traits across a wide range of taxa; this is true to the extent that failure to do so can be taken to indicate that a key trait has been omitted from the model (Roff 1986).

The timing of metamorphosis and the age of maturity have been the focus of much theoretical and empirical research. Metamorphic timing has been intensively studied in amphibians (for a review see Relyea 2007), where it is associated with the trade-offs involved in switching between very distinct aquatic and terrestrial environments. The age of maturity is usually modelled in terms of the trade-off between reproductive capacity and survival (e.g. Hochberg 1992; Abrams and Rowe 1996; Roff et al. 2005). In organisms exhibiting determinate growth, in which adult body-size is fixed at metamorphosis, metamorphic timing and age at maturity are closely interrelated. This is true of insects (Peckarsky et al. 2001), in which body-size is fixed at pupation, but not of amphibians, which lack a pupal stage.

In insects, identification of the selection pressures constraining metamorphic timing has proved problematic (Blanckenhorn 2000; Berger et al. 2006; Gotthard et al. 2007; Rimmel et al. 2011). The key difficulty is that the more obvious fitness components in adult insects are strongly and positively size-dependent, with larger males gaining a competitive advantage and larger females a fecundity advantage (Honek 1993; Blanckenhorn 2000). Hence, if larval growth is taken to be exponential, adult fitness should significantly increase with time spent in the final larval instar, implying that a short extension of the larval growth period would yield a large increase in reproductive success (Berger et al. 2006; Gotthard et al. 2007; Rimmel et al. 2011). This raises the question as to why insects do not evolve indefinitely towards ever larger body-sizes. Resolution of this paradox has focused on identifying less obvious fitness components which are negatively size-dependent (e.g. Blanckenhorn et al. 2011; Davies and Saccheri 2013), or challenging the presumed strength of the advantages associated with large size (e.g. Gotthard et al. 2007; Tammaru and Esperk 2007).

Putative viability costs of large size can be partitioned into two groups: those associated with *being* large and those associated with *becoming* large (Blanckenhorn 2000). Potential costs of being large include reduced agility, increased detectability,

time and energy costs of supporting large size and heat stress. On the other hand, the increased growth rates and/or development times required to achieve large size are hypothesized to incur costs associated with non-zero mortality rates. In particular, increased foraging activity (required for faster growth) or extended development time may increase the exposure of juveniles to predation risk. For example, predation pressure has been shown to influence life-history evolution in Trinidadian guppies *Poecilia reticulata* (Reznick et al. 1990, Abrams and Rowe 1996) and freshwater amphipods *Hyaella azteca* (Wellborn 1994). In insects, there is evidence that avian predation risk is positively size-dependent (Rommel et al. 2011); arthropod predation risk is more variable but is at least occasionally positively size-dependent also (Berger, et al. 2006; Rommel et al. 2011). Mathematical modelling suggests that predation pressure may be sufficient to balance size-dependent fecundity selection in insects when positively size-dependent, but not when size-independent (Berger et al. 2006; Rommel et al. 2011).

Parasitism also has the potential to impact life-history evolution, though often in a more subtle way than predation, since parasites do not always kill their hosts, and even when they do death is delayed (Hochberg et al. 1992). For example, infection by trematodes leads to castration in marine gastropods, and this has been correlated with life-history shifts in size and age at maturity (Lafferty 1993; Fredensborg and Poulin 2006). In insects, rates of parasitism can be very high. Solbreck et al. (1989) showed that larger species of lygaeid bugs are more susceptible to parasitization by tachinid flies than smaller ones; in *Lygaeus equestris* higher tachinid attack rates may have been responsible for the evolution of smaller bugs in Sicily compared with north Italy. In general, however, the possible impact of parasitism on insect life-history evolution has been neglected.

In Britain, *Anthocharis cardamines* larvae are heavily parasitized by the tachinid fly *Phryxe vulgaris* Fallen. In Durham (northeast England), Courtney and Duggan (1983) found that 70 out of 172 larvae (40.7%) collected over a 4 year period were parasitized; the frequency of attack varied between 38.1% and 44.8% in different years (Fig. 1.15, Chapter 1). These observations imply that in some localities attack rates can be consistently high. In other areas attack rates may be consistently low, or vary widely among years (Fig. 1.15, Chapter 1). In general, it seems likely that the infection rate in nature regularly varies between 5% and 50% (even higher infection

rates would not be inconsistent with available data which represent *underestimates* since larvae were removed from the field *before* pupation). In Durham, infection was limited to the later larval instars (Courtney and Duggan 1983), as it is when *P. vulgaris* attacks the small white butterfly *Pieris rapae* L. (Richards 1940); extended larval life is therefore likely to be risky. The parasitization of *A. cardamines* by *P. vulgaris* is therefore a good model system to study the effects of size-dependent predation (in a general sense) on insect body-size evolution.

When attack rates exhibit high spatio-temporal variability, the ability of prey to assess and respond to varying risk levels may be important in determining their age and size at metamorphosis. For larval amphibians, mathematical models which neglect the utilization of refuges or the induction of morphological defences in response to predator cues usually predict earlier metamorphosis at smaller size in the presence of predators, whereas those which incorporate these effects predict a wider range of outcomes, including later metamorphosis at larger size (Higginson and Ruxton, 2010), as is actually observed in many cases (Benard, 2004; Relyea, 2007). *A. cardamines* larvae cannot induce morphological defences and do not have access to refugia while feeding exposed on the host-plant, so this simplifies the analysis in comparison with amphibians and aquatic (mobile) insect larvae. However, their behaviour varies at the termination of growth in the final instar, when they either vacate the host-plant immediately or remain upon it during the resting phase before contraction to the pre-pupa. Since *P. vulgaris* is very unlikely to find larvae in undergrowth away from the host-plant, specimens which leave it early effectively move to a refuge near the end of larval life. This behaviour must be taken into account when modelling the effect of parasitism on larval survivorship (Gilliam and Fraser, 1987; Lima and Dill, 1990).

A. cardamines females generally oviposit on a wide range of Brassicaceous host-plant species, although in Britain they show a strong preference for *Cardamine pratensis* and *Alliaria petiolata* (Courtney and Duggan, 1983; Chapter 1). Since host use can affect larval development rate (e.g. Benrey and Denno, 1997), it could also affect the parasitism rate, since slower growing larvae would be exposed to attack for a longer period (the slow-growth high-mortality hypothesis (Clancy and Price, 1987)). This could in turn affect the evolutionary response to parasitism; if low rates of parasitism are consistently associated with host-plant species supporting rapid

development, a shift in host utilization would produce the same outcome as a reduction in body-size (in both cases the infection rate would be depressed due to shortened exposure time), but without the associated fecundity cost. This situation, as well as the consequences of larval resting phase behaviour, is best analyzed in terms of the fitness gains (at fixed infection rates) associated with a host shift or withdrawal to a refuge.

In this Chapter, I model the potential of *P. vulgaris* to modify age and size at metamorphosis in *A. cardamines*. Specifically, I investigate whether observed infection rates are sufficient to counteract the fecundity advantage of large size in females, and whether variation in larval behaviour at the end of the final instar could be an evolutionary response to high rates of parasitism. I also model the potential of host-utilization to influence the evolutionary response to parasitism through larval growth rate.

Methods

Theoretical considerations

Gotthard et al. (2007) have shown that the fecundity benefit of large female *Pararge aegeria* butterflies is depressed by time limitation (imposed by thermal constraints) during oviposition, and that this is important in modelling body-size fitness. In nature, the realized fecundity of female *A. cardamines* in some populations is low (Courtney 1982) due to the imposition of poor weather conditions (Courtney and Duggan 1983). This raises a potential difficulty in using fecundity as a measure of fitness in this species. Courtney's work was undertaken in northern English populations over 30 years ago, and therefore pre-dates the onset of global warming. In my study population in northwest England, there have frequently been extended periods of good weather in recent seasons; this may have been true also in southern English populations 30 years ago. Moreover, Duggan (1985) reported that the percentage of mature (chorionated) eggs (as well as total egg load) increases with body-size in female *A. cardamines*, and she suggested that this could be important in marginal populations subjected to severe time constraints. Hence, total and mature egg loads are likely reliable indicators of realized fitness in good and poor weather environments, respectively. Between these two extremes, mature egg load will likely give larger sized females a head start in semi-constrained conditions, which will also

translate into a fecundity advantage. I therefore consider the effects of both total and mature egg loads on the outcome of the optimality models.

In constraining the optimality models, three important decisions must be made.

1. What is the threshold size at which larvae first become vulnerable to parasitoid attack?
2. Is the subsequent attack rate size-independent or size-dependent?
3. What is the intensity of attack?

In Durham, Courtney and Duggan (1983) found that the onset of parasitism occurs in the third larval instar (body-length 6-11 mm); more limited data from Cheshire (Appendix 7) suggest that it usually occurs at some point late in the fourth instar (body-length 11-16 mm) in my study population. It may be that the threshold size varies among populations. I therefore run two versions of the models, with parasitism commencing at the start of the fourth instar (threshold body-length = 11 mm) or at the start of the fifth instar (threshold body-length = 16 mm); comparison between these models gives a good indication of how survivorship varies with threshold size. Since there is insufficient data to resolve whether attack rates are size-independent or size-dependent, I run separate models based on each assumption. It is clear that the intensity of attack varies widely within and among populations (Courtney and Duggan, 1983; Appendix 7), so I compare two versions of the models resulting in a high (50%) and low (10%) final infection rate.

In nature, larvae exhibit exponential growth interrupted by resting (moulting) periods. Since *Phryxe* lays its eggs on the larval integument, larvae are at risk of infection in both the growth and resting phases (which would not be the case if eggs were ingested); optimality models should therefore incorporate the correct growth pattern. Tammaru and Esperk (2007) have shown that the allometric growth exponent in lepidopteran larvae is typically <1 ; the assumption that it is equal to 1, and/or the neglect of resting periods, would lead to an overestimation of larval growth and hence of the value of prolonging the larval period. I therefore measured the growth trajectories of wild larvae in order to model these parameters accurately.

It is usual to measure body-size in terms of mass. However, this metric is difficult to obtain from wild larvae and butterflies, and impossible to interpret accurately in the latter, due to changes resulting from nectaring, egg-laying and senescence. I therefore design and execute my models in terms of larval body-length and adult wing-length, which are easily obtained from and interpreted in relation to field data.

Size-fecundity relation

Abdomens from laboratory reared female *A. cardamines* were dissected shortly after emergence and the eggs removed and counted. Chorionated bottle-shaped eggs were classified as 'mature'; all remaining eggs as 'immature'. Forewing lengths were measured with digital callipers. Larval lengths were measured daily through the 5th instar and the maximum value was taken to indicate mature larval size.

Larval growth parameters

A. cardamines larvae (N = 168) were located on four Brassicaceous host-plants (a small and large ecotype of *Cardamine pratensis* L., *Alliaria petiolata* Bieb. and *Barbarea vulgaris* R. Br.) in Dibbinsdale Nature Reserve over a six year period (2008-2013). Plants were revisited and larvae measured on a daily basis through the 5th instar. Fifth instar period was taken to extend from the day after the final larval ecdysis to the day before the larva was observed to have vacated the host. Mature larval-length was taken to be the maximum value in the sequence of daily measurements. Some larvae (N = 100) were measured daily from egg-hatching to vacation of the host-plant, enabling larval growth curves to be constructed from the average value of the daily measurements; these data were also utilized in mathematical models extending over the 4th and 5th instars.

The methods used to obtain the relationship between adult wing-length and mature larval-length have been described in Chapter 2; I use the equation obtained for females here ($W = 0.44L + 7.02$).

Mathematical models

The data relating wing-length, larval-length, and 5th instar period, and the size-fecundity relationship, allow the constants a , b , c , d , e and f to be determined in the following equations:

$$W = aL + b \quad (4.1)$$

$$T = cL + d \quad (4.2)$$

$$E = eW + f \quad (4.3)$$

where W = wing-length, L = (final) larval-length, T = 5th instar period, and E = egg load. My aim is to incorporate the information contained in these equations into expressions describing how fitness varies with wing-length in female *A. cardamines*. Since larvae undergo exponential growth interrupted by resting phases before each ecdysis, then, restricting attention for the moment to models in which infection is restricted to the final (5th) instar:

$$L_e = D \exp(Ct) \quad (4.4)$$

$$L_{5r} = \frac{T - d}{c} \quad (4.5)$$

where L_e = larval-length during exponential growth phase at time t , and L_{5r} = larval-length during the 5th instar resting phase (= final length); C and D are new constants to be determined.

Let

S_t = instantaneous daily survival-rate at time t

S_T = survivorship at T

'Survival-rate' here refers to the probability of avoiding parasitism; although parasitized larvae do not die immediately, their fate is determined the moment they are stung. 'Survivorship' refers to the proportion of larvae which have avoided parasitism at the end of the 5th instar. The instantaneous daily survival-rate, which determines the exponential rate at which the number of unstung larvae declines, can be modelled as a power function of larval-length at time t (L_t):

$$S_t = e^{-m_t} = k(L_t)^x \quad (4.6)$$

where k and x are constants to be determined; m_t is the instantaneous mortality per day at time t . The alternative assumptions that the attack rate is size-independent or size-dependent can be met by setting x equal to zero or to some finite value,

respectively. Assuming for the moment that x is finite (size-dependent predation), then

$$-m_t = \ln(k(L_t)^x)$$

Now to a first approximation

$$S_T = e^{-m_{T/n} \cdot \Delta\tau} \cdot e^{-m_{2T/n} \cdot \Delta\tau} \dots e^{-m_{nT/n} \cdot \Delta\tau} = \prod_{T/n}^T e^{-m_t \cdot \Delta\tau}$$

when T is divided into n finite intervals of equal duration $T/n = \Delta\tau$. Hence

$$\ln(S_T) = \sum_{T/n}^T -m_t \cdot \Delta\tau = \sum_{T/n}^T \ln(k(L_t)^x) \Delta\tau = \sum_{T/n}^T \ln(k) \cdot \Delta\tau + x \sum_{T/n}^T \ln(L_t) \cdot \Delta\tau$$

The approximation is made exact by taking the limit as $n \rightarrow \infty$ and $\Delta\tau \rightarrow dt$:

$$\ln(S_T) = \int_0^T \ln(k) dt + x \int_0^T \ln(L_t) dt = T \ln(k) + x \int_0^{\alpha T} \ln(L_e) dt + x \int_{\alpha T}^T \ln(L_{sr}) dt \quad (4.7)$$

where αT is the time at which the larva switches from the exponential to the resting phase. This equation can be solved by substituting equations (4.4) and (4.5) for L_e and L_{sr} and making use of the fact that the instantaneous daily survival-rate is 100% at day zero (just before the commencement of parasitization), i.e.

$$S_{t=0} = k(L_0)^x = k(D \exp[C0])^x = k(D)^x = 1$$

Hence

$$\ln(k) = -x \ln(D) \quad (4.8)$$

The full derivation is given in Appendix 8; the result is

$$\ln(S_T) = x \left(\frac{C\alpha^2}{2} T^2 + (1-\alpha) T \ln \left(\frac{T-d}{cD} \right) \right) \quad (4.9)$$

This equation contains four unknowns: α , C , D , and x . Since D is the size of the larva at $t = 0$ (i.e. at the commencement of the 5th instar), it can be obtained from empirical observation. I shall adopt the procedure of setting α to the average observed value of the relative duration of the exponential growth phase within the

final instar. The constant C may be determined by setting $L_e = L_{5r}$ at $t = \alpha T$, so from equations (4.4) and (4.5)

$$\frac{T-d}{c} = D \exp(C\alpha T)$$

$$C = \frac{1}{\alpha T} \ln\left(\frac{T-d}{cD}\right) \quad (4.10)$$

This value can be checked against the average value of C obtained from larval growth curves to ensure it is reasonable. To solve for x , S_T can be set to a predetermined quantity for a specified value of T in equation (4.9). I shall adopt the procedure of varying the final rate of parasitization for a larva which gives rise to an average sized female (wing-length = 20 mm), for which $T = 7.56$ days; hence, I vary the assumed value of $S_{7.56}$ in the models. Having obtained the value of x , equation (4.9) can be used to examine how larval survivorship (S_T) varies with the duration of the fifth instar (T) and the proportion of time spent in the exponential phase (α). (A separate value of C is obtained from equation (4.10) for each value of T and α .) Since T is related to larval-length (equation 4.2), and larval-length is related to wing-length (equation 4.1), S_T can be converted into the survivorship corresponding to a specific adult wing-length (S_W). The procedure can then be repeated for different values of x obtained by varying the assigned value of S_T for a fixed value of T (in my case $S_{7.56}$) in equation (4.9).

When larvae are vulnerable to parasitization during the final two instars, equation (4.7) becomes

$$\ln(S_T) = (M+T)\ln(k) + x \int_0^{\beta M} \ln(L_e)dt + x \int_{\beta M}^M \ln(L_{4r})dt + x \int_M^{M+\alpha T} \ln(L_e)dt + x \int_{M+\alpha T}^{M+T} \ln(L_{5r})dt$$

where βM and $M+\alpha T$ are the respective times of transition from the exponential to resting phases in the fourth and fifth instar, L_{4r} and L_{5r} are the larval lengths during the respective resting phases, and M is the time of moulting between the instars. For convenience, we splice out the fourth instar resting phase from the growth period; hence the second and fourth terms on the RHS can be combined to give

$$\ln(S_T) = (M + T)\ln(k) + x \int_0^{\beta M + \alpha T} \ln(L_e) dt + x \int_{\beta M}^M \ln(L_{4r}) dt + x \int_{M + \alpha T}^{M + T} \ln(L_{5r}) dt \quad (4.11)$$

Substituting equations (4.4), (4.5) and (4.8) for L_e , L_{5r} and $\ln(k)$, solving and rearranging (Appendix 8) leads to

$$\ln(S_T) = x \left(\frac{C(\beta M + \alpha T)^2}{2} + (1 - \beta)M \ln\left(\frac{L_{4r}}{D}\right) + (1 - \alpha)T \ln\left(\frac{T - d}{cD}\right) \right) \quad (4.12)$$

The constant D now represents the length of the larva at the commencement of the 4th instar, and is set to the average value obtained from empirical observation. I adopt the average observed value of α ; β is obtained from an empirically derived relationship between α and β , and M from an empirically derived relationship between M and β . To determine C , set $L_e = L_{5r}$ at $t = \beta M + \alpha T$; substituting equations (4.4) and (4.5) and rearranging gives

$$C = \frac{1}{(\beta M + \alpha T)} \ln\left(\frac{T - d}{cD}\right) \quad (4.13)$$

The length of the larva in the 4th instar resting phase (L_{4r}) can then be derived from

$$L_{4r} = D \exp(C\beta M) \quad (4.14)$$

The parameter x is solved for an assigned value of S_T (in my case $S_{7.56}$) in equation (4.12); this equation is then used to examine how larval survivorship varies with T and α (with the corresponding values of β , M , C and L_{4r}); larval survivorship at T is converted into adult survivorship at W (S_W). This procedure is then repeated for different values of x associated with changing infection-rates (assigned values of S_T).

For the size-independent model, $x = 0$ and hence equation (4.7) becomes

$$\ln(S_T) = T \ln(k) \quad (4.15)$$

The constant k is solved for an assigned value of S_T (in my case $S_{7.56}$), and is then used to calculate the survivorships of different sized larvae (which vary in the total time (T) they are exposed to attack); these are then converted into the survivorships corresponding to different adult wing-lengths (S_W). Since survival-rate is independent of larval length, there is no need to partition the vulnerable period into separate instars characterized by growth and resting phases; models in which parasitism is

restricted to the 5th instar or extends over the 4th and 5th instars differ only in the length of time larvae are exposed to attack (T or $M + T$ respectively).

The fitness (F^W) of different sized (wing-length, W) females in all models is taken to be proportional to the product of their survivorship and fecundity:

$$F^W = S_W \cdot E_W \quad (4.16)$$

where the dependency of egg load (E) on wing-length (equation 4.3) has been made explicit by introducing the subscript W . Since we are only interested in relative fitness, the constant of proportionality has been omitted. The fittest wing-length can be obtained in theory by differentiating this equation with respect to W and setting the result equal to zero, but since this produces an equation which cannot be solved analytically for W , I obtained the fittest wing-length directly from F^W by iteration.

To obtain the relative fitness gain or loss (fitness differential) resulting from varying parameters associated with the same sized specimens (e.g. the duration of the larval resting phase) note that

$$\ln(F^W) = \ln(S_W) + \ln(E_W)$$

Since fecundity (E_W) is determined by the size of the specimens, it will not impact the *change* in logarithmic fitness, which is therefore given by

$$\Delta \ln(F^W) = \ln(F'^W) - \ln(F^W) = \ln\left(\frac{F'^W}{F^W}\right) = \Delta \ln(S_W)$$

where the prime denotes the altered fitness value. Hence the fitness differential resulting from varying parameters independently of size (wing-length) is given by

$$\frac{F'^W}{F^W} = \exp(\Delta \ln(S_W)) \quad (4.17)$$

Note that for the size-independent models the change in fitness resulting from a change in larval period is given by

$$\Delta \ln(S_T) = (T' - T) \ln(k)$$

Therefore when T' differs from T due to factors unrelated to size (e.g. α ; see equation (4.18) below), $T' - T$ lacks a term in L (larval-length) which is ultimately related to

W (wing-length). In such cases, $\Delta \ln(S_T)$ and hence the fitness differential is constant with wing-length. This is not the case for the size-dependent models, due to the presence of cross-terms between L and factors unrelated to size due to the squaring of T in equations (4.9) and (4.12).

Results

Size-fecundity relation

Laboratory reared female *A. cardamines* exhibited a strong size-fecundity relationship. This was the case irrespective of whether size was measured by adult wing-length or larval body-length, or whether fecundity was measured by total egg number or mature egg number (Fig. 4.1). The results with mature egg number confirm that even if females are unlikely to lay their full complement of eggs, larger specimens will gain a 'head start' advantage by carrying a greater number of eggs which can be laid immediately after mating (which takes place shortly after emergence). The results with larval-length demonstrate that the fecundity advantage of large adults is gained through the size attained in the final larval instar. In contrast with the results obtained by Duggan (1985), the percentage of mature eggs does not change significantly with wing-length (Fig. 4.2); hence the proportional allocation of resources to capital breeding (the use of stored energy for reproduction (Stearns, 1992)) is constant with body-size in the study population.

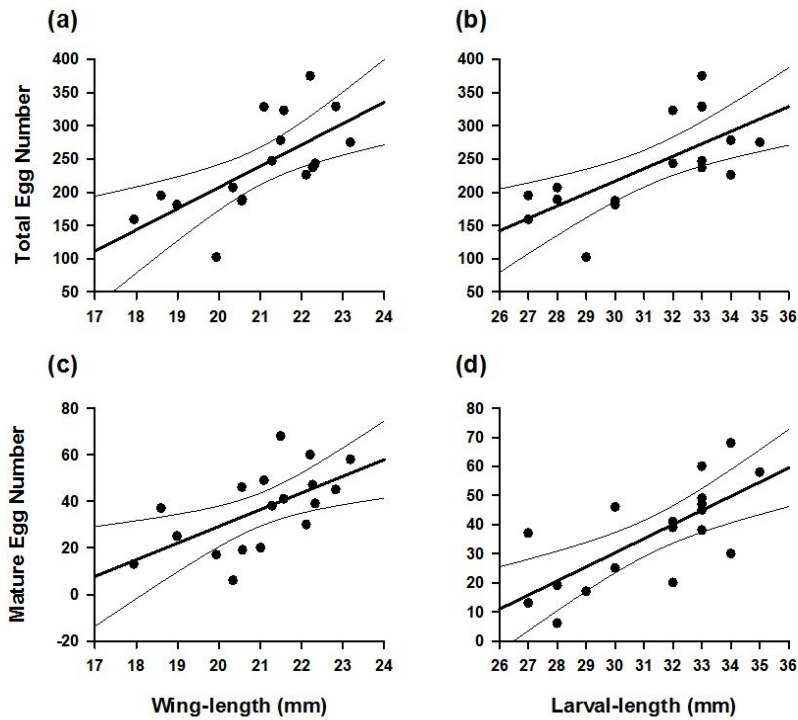


Figure 4.1 *A. cardamines* size-fecundity relationship with 95% confidence bands (N = 17), from laboratory reared larvae. Two measures of size and fecundity are given: adult wing-length (left column) and larval body-length (right column); total egg number (top row) and mature egg number (bottom row). Regression equations for the best-fit lines are: Total E = $32.0W - 433$ ($p = 0.003$); Mature E = $7.2W - 114$ ($p = 0.008$); Total E = $18.7L - 343$ ($p = 0.002$); Mature E = $4.8L - 115$ ($p = 0.0008$); where E = egg number, W = wing-length, L = larval-length, p = significance of regression line slope.

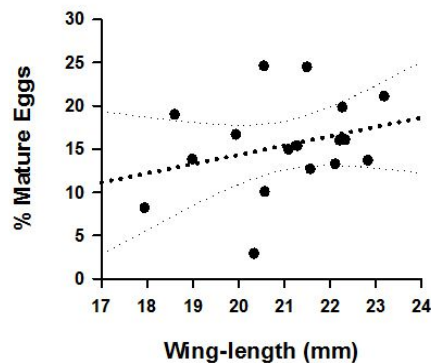


Figure 4.2 Percentage of mature *A. cardamines* eggs against wing-length, with 95% confidence bands (% Mature E = $0.011W - 0.072$, $R^2 = 0.025$, $p = 0.25$, N = 17).

Larval growth-curves

Wild larvae which were measured daily from hatching to maturity showed a characteristic interrupted exponential growth-curve on the three principal hosts, small *C. pratensis*, large *C. pratensis* and *A. petiolata* (Fig. 4.3). The interruptions or

resting periods occurred at the end of each instar, and are characterized by (near) cessation of growth. When the resting phases are spliced out of log-transformed versions of the growth-curves (Fig. 4.3), the resultant straight lines confirm that wild specimens can (on average) be taken to grow exponentially. The key assumption in the mathematical models concerning the partitioning of each instar into an exponential growth phase followed by a resting phase is based on these empirical data.

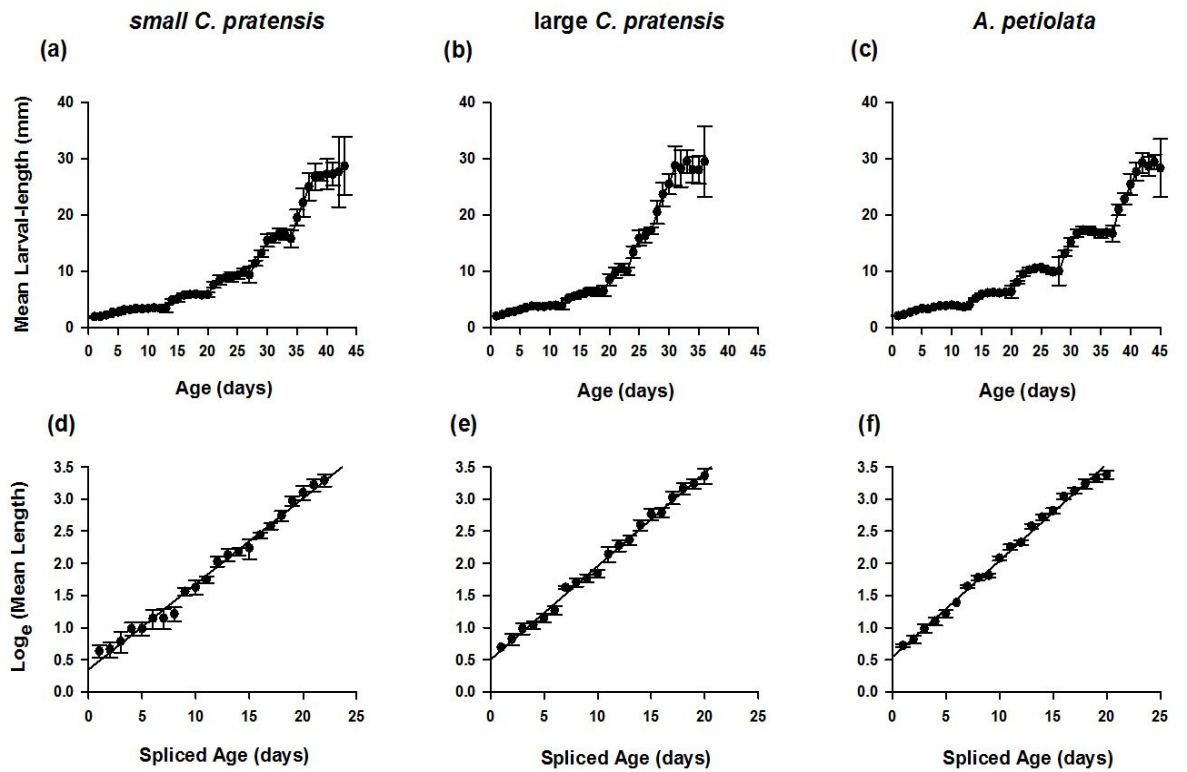


Figure 4.3 Growth-curves obtained from wild *A. cardamines* larvae. Each data point represents the average measurement (\pm 95% CI) from a cohort of larvae from eggs laid at the same sub-site in the same year. Top: full growth-curves showing mean size (length) with age on (a) small *C. pratensis* at Otters' Tunnel in 2009 ($N_1 = 17$, $N_5 = 6$), (b) large *C. pratensis* at Upper Tip in 2009 ($N_1 = 24$, $N_5 = 6$), (c) *A. petiolata* at Boden's Hey in 2009 ($N_1 = 59$, $N_5 = 17$) (where N_1 = number of larvae entering 1st instar, N_5 = number entering 5th instar). The step-like pattern indicates that exponential growth is interrupted by resting phases. The curves are semi-schematic insofar as individual larvae remained in the resting phases for different periods; to keep mean lengths synchronized, all larvae exiting a resting phase were reassigned to the average age at which exponential growth resumed. Bottom: log-transformed growth-curves from which the resting phases have been spliced out. Regression equations: (d) $\ln L = 0.13A + 0.35$ (small *C. pratensis*); (e) $\ln L = 0.15A + 0.50$ (large *C. pratensis*); (f) $\ln L = 0.15A + 0.53$ (*A. petiolata*); L = mean length, A = spliced age.

Relative duration of exponential growth phase

The relative duration of the exponential growth phase in the final instar (α) was bimodal in wild larvae (Fig. 4.4a). Larvae either lacked a resting phase ($\alpha = 1$), or the

period spent in the growth phase was symmetrically distributed around a mean value of $\alpha = 0.6$. Since α is a proportion, the observed bi-modality is unlikely to be due to predation, and this can be confirmed analytically (Appendix 9). However, this does *not* imply that some larvae actually lack a *physiological* resting phase; since the observations were restricted to the time spent on the host-plant, they reveal contrasting behaviours at the end of the growth phase, whereby some larvae remain on the host for the resting period and others leave it immediately. This is corroborated by the growth of larvae in the 4th instar, none of which lacked a resting phase (Fig. 4.4b). In the 5th instar, larvae were observed to omit the resting phase on all host-plants (except *B. vulgaris*, which was relatively under-sampled) and this trait varied in frequency among years, being significantly commoner ($\chi^2 = 17.1$, $df = 1$, $p = 0.00004$) in 2013 than in 2008-11 (Fig. 4.4c,d). Since these larvae belong to a distinct behavioural sub-population, they are treated separately in the analyses of the predictors of the 4th and 5th instar periods.

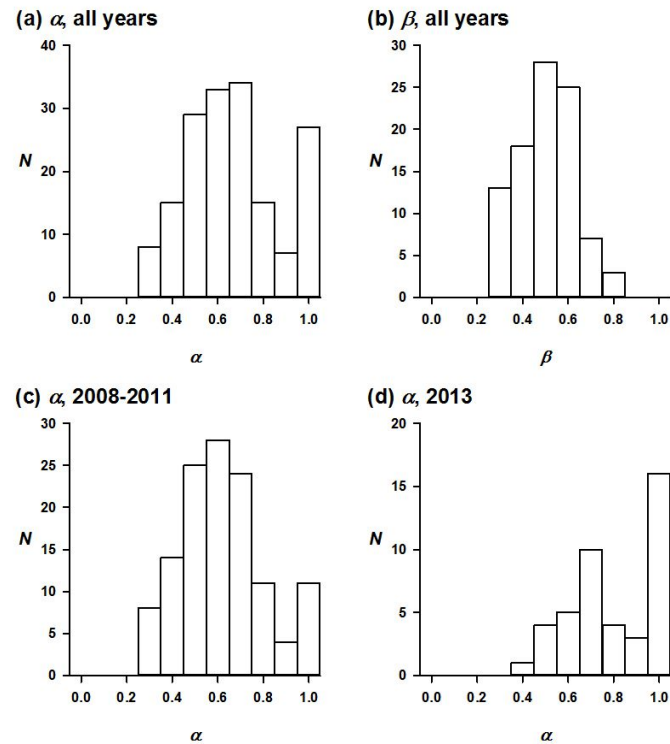


Figure 4.4 Histograms showing the distribution of the proportion of time spent in the exponential growth phase. (a) Distribution of proportion of time spent in growth phase in 5th instar (α), exhibiting bi-modality ($N = 168$); (b) Distribution of proportion of time spent in growth phase in 4th instar (β), lacking bi-modality ($N = 94$); (c) Distribution of α in the years 2008-2011 ($N = 125$); (d) Distribution of α in 2013 ($N = 43$).

Predictors of 4th and 5th larval instar period

Regression analysis demonstrated that larval-length, relative duration of the exponential phase (α) and large *C. pratensis* had highly significant effects on 5th instar duration (T), but that the other host-plants and year did not (Table 4.1). The collinearity statistics for the final regression model indicate that larval-length, α and large *C. pratensis* act independently on 5th instar duration. Hence, larval-length can be varied independently of any specified value of α and of host use in the mathematical models. This means that the last three terms in the regression equation

$$T = 0.305L - 5.468\alpha - 0.961K_L + 1.843 \quad (4.18)$$

can be combined into the constant d in equation (4.2). (K_L is a dummy variable equal to 1 when larvae are feeding on large *C. pratensis* and 0 otherwise.) For example, if we set $\alpha = 0.6$ (the average value) and assume that the larvae are not feeding on large *C. pratensis* ($K_L = 0$), then $d = -1.438$ in equation (4.2); whereas if we assume that the larvae are feeding on large *C. pratensis* ($K_L = 1$), then $d = -2.399$.

Table 4.1 Regression analysis on the effects of larval-length (L), relative duration of the exponential growth phase (α), and host-plant on wild *A. cardamines* 5th larval instar duration. Initially, all host-plants (small and large *C. pratensis*, *A. petiolata* and *B. vulgaris*) and study years (2008-11 and 2013) were included in the analysis (separately as dummy variables); after backward deletion, only large *C. pratensis* (K_L) remained.

	Unstandardized Coefficients		Standz'd Coeffs	t	Sig.	Correlations			Collinearity Statistics	
	B	S.E.	Beta			Zero-order	Partial	Part	Tol	VIF
Const.	1.843	2.087		.883	.379					
<i>L</i>	.305	.065	.327	4.685	<.001	.347	.372	.326	.993	1.007
α	-5.468	.922	-.413	-5.929	<.001	-.441	-.452	-.412	.994	1.006
K_L	-.961	.322	-.208	-2.980	.003	-.201	-.247	-.207	.997	1.003

Dependent variable: 5th instar duration (T). N = 141, F = 23.28, adjusted $R^2 = 0.323$, $P < 0.001$.

Regression analysis showed that the proportion of time spent in the exponential growth phase (β) and large *C. pratensis* had highly significant effects on the duration (M) of the 4th instar (Table 4.2):

$$M = -6.548\beta - 2.246K_L + 9.808 \quad (4.19)$$

M was not related to T directly, but there was a significant relationship ($p = 0.015$) between β and α :

$$\beta = 0.227\alpha + 0.376 \quad (4.20)$$

Hence, it is always possible to assign a value of M to any value of T in the models through α and K_L via equations (4.18), (4.20) and (4.19).

Table 4.2 Regression analysis on the effects of the relative duration of the exponential phase (β) and large *C. pratensis* (K_L) on wild *A. cardamines* 4th larval instar duration. The table shows the final model after backward deletion of 4th instar final larval-length, other host-plants and year.

	Unstandardized Coefficients		Standzd Coeffs	t	Sig.	Correlations			Collinearity Statistics	
	B	S.E.	Beta			Zero-order	Partial	Part	Tol	VIF
Const	9.808	.799		12.269	<.001					
β	-6.548	1.523	-.426	-4.299	<.001	-.396	-.450	-.424	.994	1.006
K_L	-2.246	.610	-.365	-3.682	<.001	-.330	-.396	-.363	.994	1.006

Dependent variable: 4th instar duration (M). N = 76, F = 14.85, adjusted $R^2 = 0.270$, $P < 0.001$.

For the specific sub-population characterized by $\alpha = 1$ (no resting phase on the host-plant), regression analysis showed that larval-length, as well as both small and large *C. pratensis*, had highly significant effects on the duration of the 5th instar (Table 4.3). The effect of the two *Cardamine* ecotypes (in comparison with *A. petiolata*) is almost the same, so we can substitute their average effect into the regression equation as a single term (K):

$$T = 0.300L - 1.365K - 3.861 \quad (4.21)$$

The value of T obtained from this equation is substituted into equations (4.9), (4.12) and (4.15) when studying the susceptibility of this sub-population to parasitism; in setting $\alpha = 1$ in the former two expressions, the effect of the resting phase is automatically excluded in the size-dependent models. The data are too sparse to draw any definite conclusions as to how these larvae behave in the 4th instar; for the purposes of mathematical modelling, I therefore assume that they behave in the same way as the average phenotype with $\alpha < 1$; i.e. with the values of β and M corresponding to the assumption that $\alpha = 0.6$.

Table 4.3 Regression analysis on the effects of larval-length (L), small *C. pratensis* (K_S) and large *C. pratensis* (K_L) on wild *A. cardamines* 5th instar duration for larvae with $\alpha = 1$ (after backward deletion of all years from the model).

	Unstandardized Coefficients		Standzd Coeffs	t	Sig.	Correlations			Collinearity Statistics	
	B	S.E.	Beta			Zero-order	Partial	Part	Tol	VIF
Const	-3.861	2.483		-1.555	.134					
L	.300	.087	.525	3.459	.002	.509	.585	.493	.884	1.132
K_S	-1.473	.508	-.478	-2.897	.008	-.071	-.517	-.413	.747	1.339
K_L	-1.257	.388	-.525	-3.238	.004	-.440	-.560	-.462	.775	1.291

Dependent variable: 5th instar duration (T). $N = 27$, $F = 8.72$, adjusted $R^2 = 0.471$, $P < 0.001$.

Mathematical modelling parameters

From the regression equation for wing-length on larval-length (females), $a = 0.44$, $b = 7.02$; from the regression equation (4.18) for the predictors of 5th instar period, $c = 0.305$, with d varying according to α and host use as described above; and from those of egg load on wing-length (Fig. 4.1), $e = 32$, $f = -433$ (total eggs) or $e = 7.2$, $f = -114$ (mature eggs). For size-dependent models in which parasitism extends over the 4th and 5th instars, $D = 11$ (corresponding to the average length of wild larvae entering the 4th instar); for consistency, in models in which parasitism was restricted to the 5th instar, D was set to the length of larvae at the commencement of that instar giving rise to average sized adults (wing-length = 20 mm) in models extending over the 4th and 5th instars (16.68 mm, in reasonable agreement with empirical measurements (average ≈ 18 mm)). The value of C , obtained from equations (4.10) and (4.13), tended to vary around 0.13, in good agreement with values derived from growth measurements of wild larvae (0.13/0.15, see Fig. 4.3).

In standard models, $\alpha = 0.6$ (the average value of the proportion of time spent in the exponential growth phase (Fig. 4.4a) during the 5th instar (neglecting cases where $\alpha = 1$)); for models in which the resting phase was extended/reduced, α was varied between 0.3 and 0.9 (the lowest and highest observed values (Fig 4.4a)), and for those in which the resting phase was removed, $\alpha = 1$. For models extending over 4th and 5th instars, M was derived from β using equation (4.19), β itself having been derived from the value of α using equation (4.20); for standard models, $\alpha = 0.6$, $\beta = 0.51$ and $M = 6.45$. These values of β and M were retained in models for which $\alpha = 1$.

The values of k and x , derived from equations (4.8), (4.9), (4.12) and (4.15) for different assumed rates of infection (S_T) at the end of the final instar of an average

sized larva ($T = 7.56$, $C = 0.126$, $\alpha = 0.6$, $L_{4r} = 16.68$, $M = 6.45$, $\beta = 0.51$), varied with the model assumptions and infection rate (Table 4.4). For the size-independent models, k represents the daily survival-rate ($x = 0$ in equation (4.6)), and hence decreases with increasing infection rate (decreasing survivorship) and when the allowed infection period is shorter (i.e. when parasitism is restricted to the 5th instar). For the size-dependent models, the value of k and the (absolute) value of x increases when parasitism is restricted to the 5th instar and when survivorship decreases. The behaviour of x , which determines the rate at which susceptibility to infection increases with larval length, reflects the fact that when the allowed infection period is shorter or final survivorship is lower, the parasites must work harder to achieve the required infection rates.

Table 4.4 Derived values of the parameters k and x in size-independent and size-dependent mathematical models, showing their behaviour when survivorship at the end of the 5th instar of an average sized larva ($S_{7.56}$, inversely related to parasitization rate) varies, and when parasitism extends over the 4th and 5th instars or is restricted to the 5th instar.

$S_{7.56}$	4th + 5th instar			5th instar		
	Size-Independent	Size-Dependent		Size-Independent	Size-Dependent	
	k	k	x	k	k	x
0.9	0.992507	1.031450	-0.01291	0.986159	1.103200	-0.03490
0.8	0.984198	1.067780	-0.02735	0.970913	1.231227	-0.07392
0.7	0.974861	1.110518	-0.04372	0.953913	1.394432	-0.11816
0.6	0.964193	1.161988	-0.06261	0.934659	1.609919	-0.16922
0.5	0.951726	1.225950	-0.08496	0.912386	1.908163	-0.22962
0.4	0.936686	1.309046	-0.11231	0.885847	2.349382	-0.30354
0.3	0.917647	1.424539	-0.14757	0.852769	3.071988	-0.39885
0.2	0.891468	1.604825	-0.19726	0.808235	4.483004	-0.53317
0.1	0.848433	1.967436	-0.28222	0.737422	8.554304	-0.76279

The assumption that larval survival-rate is dependent on size leads to an increase in the differential selection pressure operating on different sized adults. Specifically, for any assumed value of $S_{7.56}$, smaller specimens do relatively better and larger ones relatively worse than in models for which survival-rate is size-independent (Fig. 4.5). The size-dependent model approximates more closely to the size-independent one when attack rates are low ($S_{7.56}$ high), since x tends to zero (Table 4.4) and the size-dependency diminishes (Fig. 4.5).

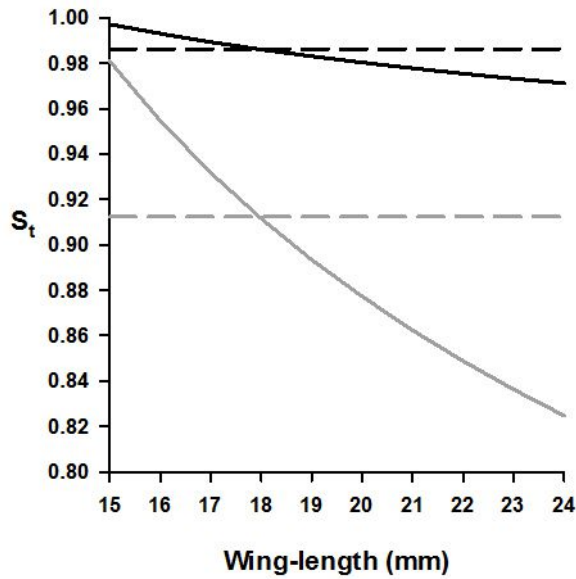


Figure 4.5 Instantaneous daily survival-rate (S_t) at the termination of the 5th larval instar for different sized (wing-length) adult *A. cardamines* when parasitoid attack-rate is size-dependent (solid lines) and size-independent (dashed lines). Models in which the final survivorship is high ($S_{7.56} = 0.9$) and low ($S_{7.56} = 0.5$) are shown in black and grey respectively; in both cases, parasitism is restricted to the 5th instar.

Fitness vs. wing-length

Standard models ($\alpha = 0.6$, $\beta = 0.51$, $M = 6.45$) of fitness (F^W) with wing-length demonstrate that increased exposure to parasitism reduces the rate of gain in fitness with body-size (Fig. 4.6); at high attack rates, the fecundity advantage of large size is balanced by infection risk and the fitness curves tend to level-off. The effect of parasitism is weakest when the attack rate is size-independent and the infection period extends over the 4th and 5th instars; in this case, the highest infection rate observed in the field (corresponding to $S_{7.56} = 0.5$) is insufficient to prevent fitness increasing with size in an approximately linear fashion over the observed wing-length range for the species, although the rate of increase in fitness is greatly reduced compared with lower infection rates. On the other hand, the effect of parasitism is strongest when the attack rate is size-dependent and the infection period is restricted to the 5th instar; the highest observed infection rate then produces a weakly curved fitness function due to the trade-off between fecundity and infection risk. In this case the fittest wing-length (19.9 mm) in the model for which fecundity is measured by total egg load (Fig. 4.6a) is close to the female average (20.0 mm) for the study area. The effect of parasitism is slightly weaker in models in which fecundity is measured

by mature egg load (Fig. 4.6b); however, this difference is not sufficient to support the conclusion that parasitism is less effective in time-constrained environments.

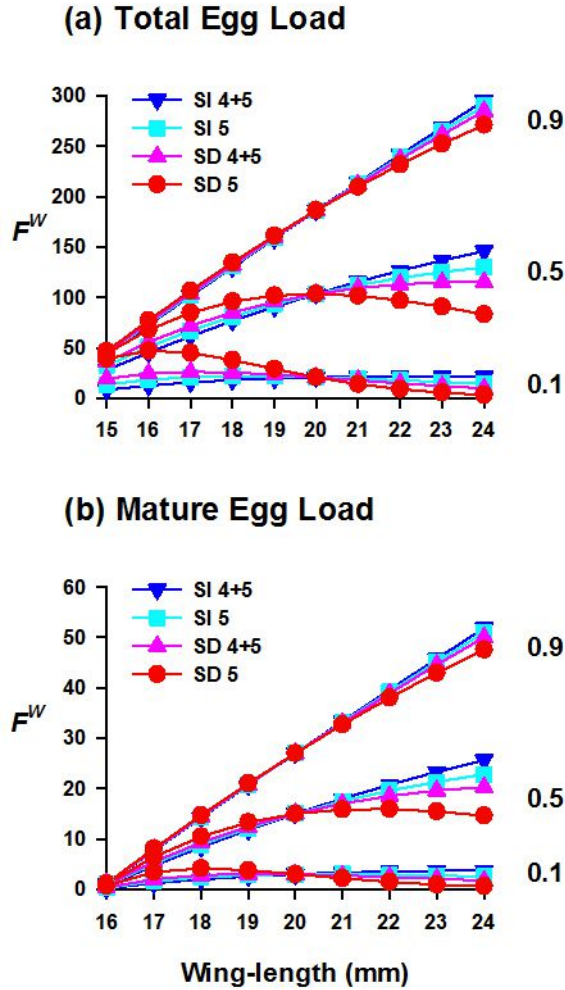


Figure 4.6 Size-fitness curves for female *A. cardamines* derived from size-independent (SI) and size-dependent (SD) models in which parasitization is limited to the fifth larval instar (5) or extends over the fourth and fifth instars (4+5) and fitness (F^W) is measured by (a) total egg load or (b) mature egg load. In each case, the fitness curves are shown for the three levels of survivorship ($S_{7.56} = 0.9, 0.5$ and 0.1) indicated at right.

Combined effect of size and resting phase duration

The impact of resting phase duration ($1 - \alpha$) on fitness was analysed by varying α in equation (4.18). The values of x derived in the standard models were retained in order to examine the effect of resting phase duration in an environment with unchanging attack rates.

The fitness surface for the combined effect of size and resting phase duration at a low infection rate ($S_{7.56} = 0.9$) is planar in all models (similar to Fig. 4.7c, but steeper);

fitness increases rapidly with wing-length (since the size-fecundity relationship overwhelms infection risk) and is relatively unaffected by resting-phase duration. At the highest observed infection rate ($S_{7.56} = 0.5$), the shape of the fitness surface varies with model assumptions (surfaces for models in which fitness is measured by total egg load are shown in Fig. 4.7; models in which fitness is measured by mature egg load give similar results). For the model in which parasitoid attack is size-independent and the infection period is long (4th plus 5th instars) the surface is still planar (Fig. 4.7c), although the fitness plane rises less steeply with wing-length than in the corresponding low infection rate model. Fitness is predominantly determined by size; contours of equal fitness occur in bands running nearly parallel to lines of equal wing-length (wing-length isolines) so that fitness changes slowly with resting period (along these isolines) but quickly with wing-length (along resting period isolines). On the other hand, the fitness surface is saddle shaped (Fig. 4.7b) for the model in which parasitoid attack is size-dependent and the infection period is short (5th instar only). Fitness is predominantly determined by resting phase duration; the fitness contours are strongly curved, such that fitness changes relatively quickly with resting period (along the wing-length isolines) but slowly with wing-length (along the resting period isolines). Shorter resting periods are strongly favoured. Models in which parasitoid attack is size-independent but the resting period short (Fig. 4.7a) or parasitoid attack is size-dependent but the resting period long (Fig. 4.7d) produce curved surfaces intermediate between these extremes.

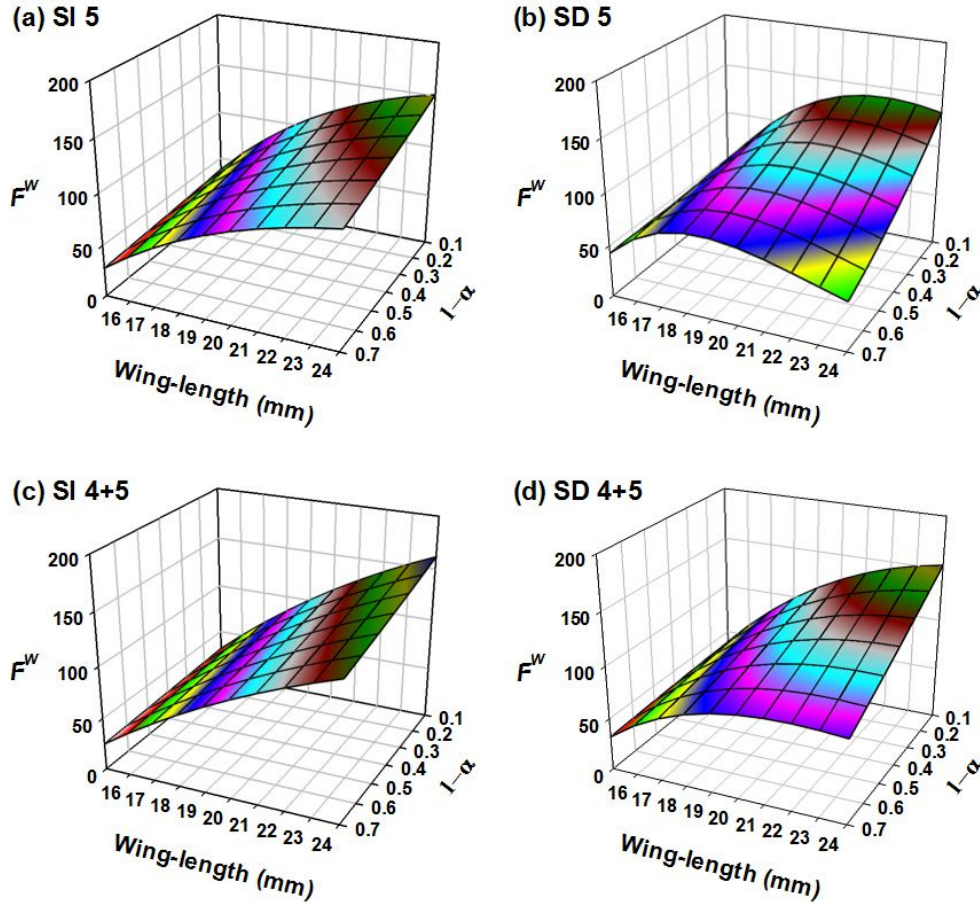


Figure 4.7 Fitness surfaces for the combined effect of size (wing-length) and resting phase duration ($1 - \alpha$) on female *A. cardamines* fitness (F^w , measured by total egg-load) at a high ($S_{7.56} = 0.5$) infection rate. Model coding: SI = size-independent parasitoid attack-rate, SD = size-dependent attack-rate, 4+5 = parasitism extends over 4th and 5th instars, 5 = parasitism restricted to 5th instar. Colours represent contours of equal fitness.

Resting phase duration impacts fitness in different ways in size-independent and size-dependent models (Fig. 4.8). In the absence of size-dependency, the change in fitness associated with a change in resting phase duration is constant with wing-length, whereas with size-dependency the fitness gains or losses increase with wing-length (the mathematical reason for this is given at the end of the Methods section). In all models, any shortening of the resting period will produce an increase in fitness provided the infection rate is finite (Fig. 4.8); in the absence of constraints, specimens will always make fitness gains by reducing exposure to the parasite. The existence of contrasting behaviours at the end of the growth phase, whereby some larvae spend the resting phase exposed on the host-plant and others do not (Fig. 4.4), strongly impacts the survivorship of larvae threatened with parasitism (Table 4.5); specimens which leave the host-plant early make large fitness gains relative to the

average fitness of those which remain on it (Fig. 4.8). For small specimens, the fitness gain associated with this behaviour is greater in the size-independent models, and for large ones it is greater in the size-dependent models; this is a consequence of the fact that small/large specimens are relatively more vulnerable to attack in the size-independent/dependent models respectively (Fig. 4.5). The fitness gain is also greater when the infection period is short (except for the smallest specimens in the size-dependent models; Table 4.5 and Fig. 4.8).

Table 4.5 Fitness differential (from equation 4.17) for early vacation of the host-plant (compared with average resting phase duration on the host plant), a host shift from *A. petiolata*/*B. vulgaris*/small *C. pratensis* to large *C. pratensis* (at average resting phase duration), and early vacation of the host-plant combined with a host shift from *A. petiolata* to small + large *C. pratensis*, for size-independent (SI) and size-dependent (SD) models in which parasitism extends over the 4th + 5th instars (4 + 5) or is restricted to the 5th instar (5) at low ($S_{7.56} = 0.9$) and high ($S_{7.56} = 0.5$) infection rates.

Comparison	Female Size	SI				SD			
		4+5		5		4+5		5	
		0.9	0.5	0.9	0.5	0.9	0.5	0.9	0.5
Early Vacation	15 mm	1.02	1.13	1.04	1.26	1.01	1.08	1.01	1.04
	24 mm	1.02	1.14	1.04	1.27	1.06	1.46	1.10	1.92
Host Shift	15 mm	1.02	1.17	1.01	1.09	1.01	1.09	1.00	1.01
	24 mm	1.02	1.17	1.01	1.09	1.03	1.21	1.02	1.14
Early Vacation + Host Shift	15 mm	1.05	1.35	1.06	1.43	1.02	1.15	1.01	1.05
	24 mm	1.05	1.36	1.06	1.44	1.09	1.74	1.13	2.19

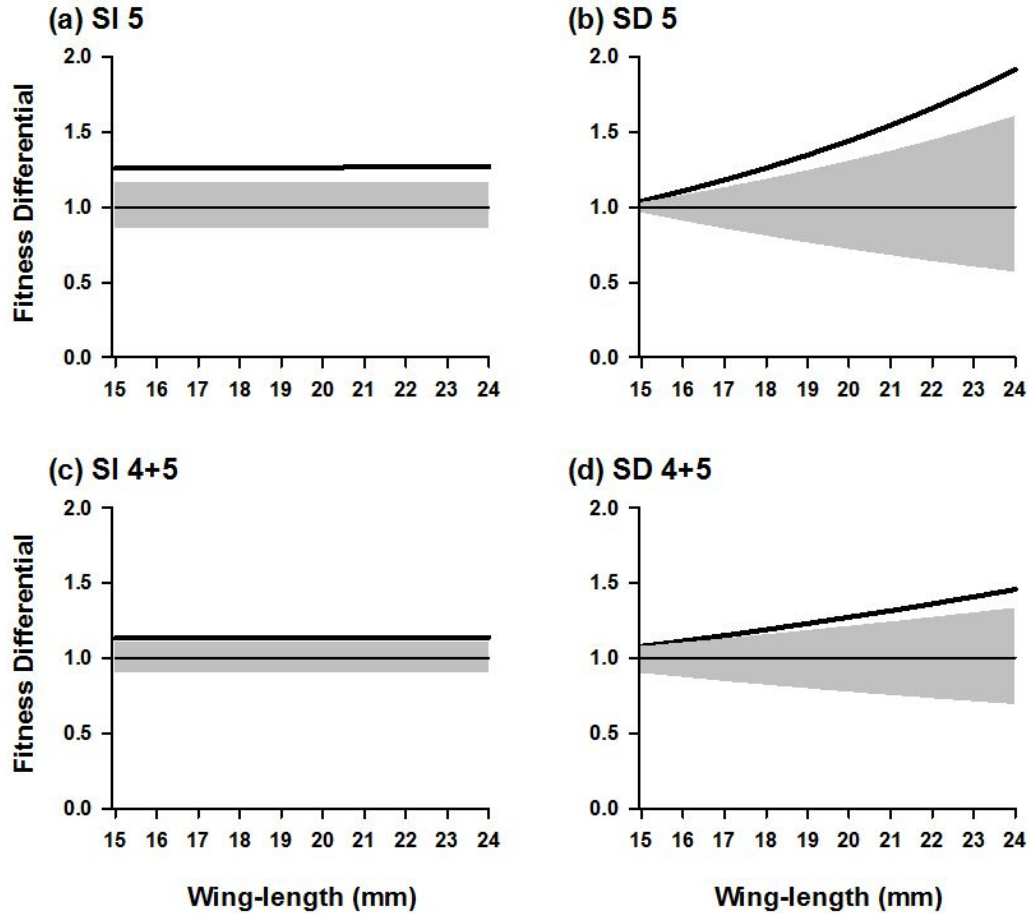


Figure 4.8 Fitness differential in relation to the duration of the resting phase at a high ($S_{7.56} = 0.5$) infection rate. The fitness of individuals with an average resting phase duration ($1 - \alpha = 0.4$) has been set to unity across all wing-lengths (thin solid horizontal line); the fitness gains or losses relative to this (from equation 4.17) are shown by the grey shaded area, which extends over the observed range of resting phase durations (from $1 - \alpha = 0.7$ at bottom to $1 - \alpha = 0.1$ at top) for specimens remaining on the host-plant, and by the thick solid line, for specimens which leave the host-plant immediately at growth termination. Model coding: SI = size-independent parasitoid attack-rate, SD = size-dependent attack-rate, 4+5 = parasitism extends over 4th and 5th instars, 5 = parasitism restricted to 5th instar.

Effect of host use

The impact of host use on fitness was analysed by changing the variable K_L in equations (4.18) and (4.19) from 0 (for *A. petiolata*, *B. vulgaris* and small *C. pratensis*) to 1 (for large *C. pratensis*); the values of x derived in the former models were retained in the latter, to study the effects of varying host use in an environment with constant attack rates. The results show that fitness always increases on large *C. pratensis* (Table 4.5 and Fig. 4.9). Interestingly, greater fitness gains are associated with models in which parasitism extends over the 4th and 5th instars; this is related to the fact that large *C. pratensis* reduces the time larvae spend in both instars (see

equations 4.18 and 4.19), leading to a greater cumulative reduction in exposure to the parasitoids. For small specimens, the relative increase in fitness is greater in the size-independent models, whereas for large ones it is greater in the size-dependent models (cf. Fig. 4.5).

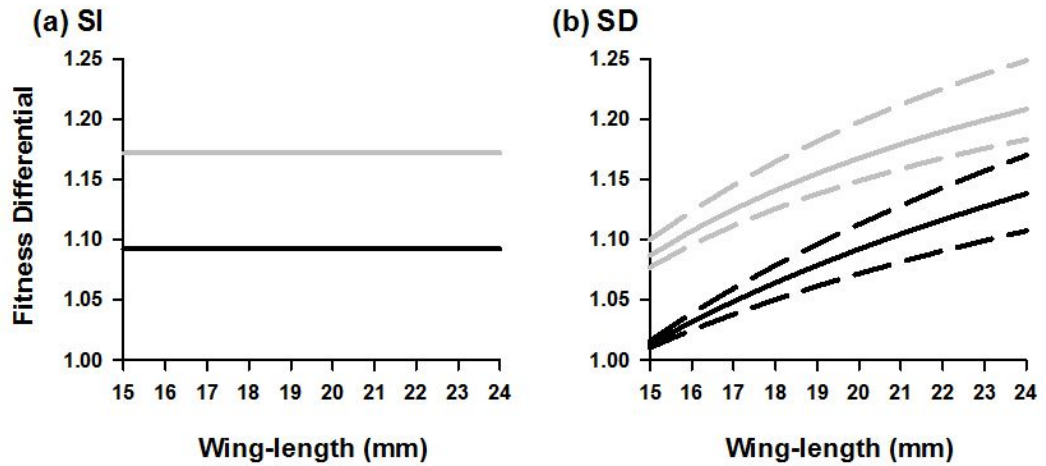


Figure 4.9 Fitness differential in relation to a host shift from *A. petiolata*/*B. vulgaris*/small *C. pratensis* to large *C. pratensis* at a high ($S_{7.56} = 0.5$) infection rate. The fitness of individuals on the former hosts has been set to unity across all wing-lengths (x-axis); the fitness gains relative to this for models in which parasitization is limited to the 5th instar are shown in black, and those for models in which parasitization extends over the 4th and 5th instars are shown in grey. (a) Size-independent models (no effect of resting period). (b) Size-dependent models with an average resting phase duration ($1 - \alpha = 0.4$) (solid lines) or with the longest ($1 - \alpha = 0.7$) and shortest ($1 - \alpha = 0.1$) observed resting phases (upper and lower dashed lines, respectively).

The combined effect of a host shift and early vacation of the host-plant were analyzed by substituting $K = 1$ into equation (4.21) and $K_L = 0$ in equation (4.19), inserting the obtained values of T and M together with $\alpha = 1$ into equations (4.9) and (4.12), and comparing the resultant fitness with that obtained by substituting $K_L = 0$ into equations (4.18) and (4.19) and inserting the obtained values of T and M together with $\alpha = 0.6$ into equations (4.9) and (4.12). In this case, the host shift is from *A. petiolata* to small and large *C. pratensis* (I have opted to neglect the effect of large *C. pratensis* in the 4th instar). The values of x were taken over unchanged from the standard models. The results show that a shift from *Alliaria* to *Cardamine* in combination with early vacation of the host-plant produces large fitness gains (Table 4.5 and Fig. 4.10). In the size-dependent models (Fig. 4.10b), there is a reversal as to which infection regime (5th instar only or 4th + 5th instars) is associated with the

highest fitness gains with size; this is related to the fact that as specimens get larger, they spend relatively more time in the 5th instar, and so the fitness benefits related to changing host and early vacation increase more quickly with size when parasitism is concentrated in that instar. For the size-independent models (Fig. 4.10a) the fitness gains are greater than in size-dependent models for small specimens but less for large ones (cf. Fig. 4.5); the fitness gain is always greater when the infection period is limited to the 5th instar.

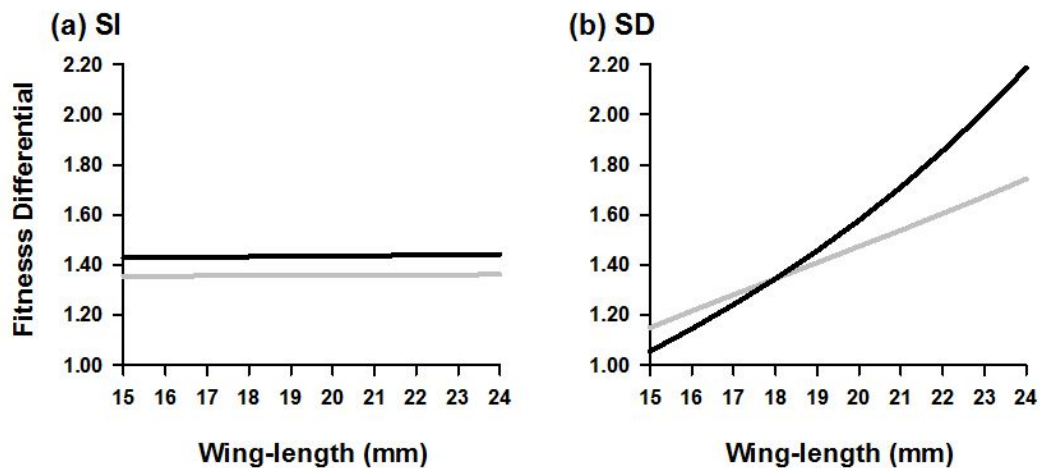


Figure 4.10 Fitness differential in relation to a host shift from *A. petiolata* to small *C. pratensis* and large *C. pratensis* combined with early vacation of the host-plant ($\alpha = 1$) at a high ($S_{7.56} = 0.5$) infection rate. The fitness of individuals on the former host and with an average resting phase duration ($1 - \alpha = 0.4$) has been set to unity across all wing-lengths (x-axis); the fitness gains relative to this for models in which parasitization is limited to the 5th instar are shown in black, and those for models in which parasitization extends over the 4th and 5th instars are shown in grey. (a) Size-independent models. (b) Size-dependent models.

Discussion

Impact of parasitism on body-size evolution

Realistic levels of parasitism are sufficient to counteract the size-fecundity relationship in size-dependent models, particularly when the allowed infection period is short (i.e. restricted to the 5th larval instar). In this case, the optimal wing-length is very close to the observed average for an attack rate (50%) which has been recorded in the study area (Fig. 4.6). However, larvae do not reach the threshold size for attack as late as the start of the fifth instar in the study population; it most likely occurs at some point in the fourth instar, while it is recorded as occurring in the third instar in Durham (Courtney and Duggan, 1983). Moreover, optimality is weak (the curvature of the fitness function is not strong) and labile (small changes in attack rate produce

large changes in optimal wing-length). Hence, stabilizing selection for a specific optimal wing-length would require very high levels of parasitism to be maintained over a very long period of time (Lande and Arnold, 1983; Kingsolver and Pfennig, 2007). Instead, prolonged exposure to fluctuating levels of parasitism will probably help to maintain the size variance in the population, while preventing the runaway evolution of ever larger body-sizes.

If parasitoid attack is not size-dependent, then only very high rates of infection (currently unobserved) can balance the size-fecundity relationship (Fig. 4.6). Nevertheless, the power of the size-fecundity relationship is diminished in the size-independent models; instead of a runaway fecundity advantage, the rate of gain in fitness with increased body-size is greatly reduced by parasitoid attack. If time-constraints limit the fitness benefit of egg-loads in excess of the currently observed maximum, then the potential advantages of large size may be negated by parasitism even in the absence of size-dependency. Again, the most likely outcome would be the imposition of an upper size limit and the maintenance of size variance, rather than strong selection for an optimal wing-length.

There is currently insufficient evidence to decide whether the attack rate of *P. vulgaris* is size-independent or size-dependent. In general, it is hypothesized that tachinids are initially attracted to their host's food-plants by olfactory (volatile chemical) cues, and thereafter locate their victims visually (Stireman et al., 2006). If visual stimulation is important in the search behaviour of *P. vulgaris*, then the attack rate is most likely size-dependent, assuming that (all else being equal) larger larvae are easier to see. Olfactory cues may also be important in locating hosts directly (Stireman et al., 2006); if larger larvae are more odoriferous this would also imply that the attack rate is size-dependent. Given the infection rates observed in the field, this would imply that *P. vulgaris* has been an important selective agent in opposing the size-fecundity relationship and maintaining small body-size in *A. cardamines*.

The current study is the first to demonstrate that predation (in a general sense) has the potential to constrain body-size evolution in a model system for which the size-fecundity relationship, wild larval growth curves (including resting periods) and predator (parasitoid) attack rates are accurately known. In general, high attack rates and size-dependency are likely to be required if predation/parasitism is to be solely

responsible for counteracting the size-fecundity relationship in insects. This supports the conclusions of Berger et al. (2006) and Rimmel et al. (2011), who developed models from less accurately parameterized systems. Furthermore, the results reported here extend the earlier findings in two important ways. Firstly, high levels of parasitism are more likely to help maintain size-variance than to induce strong stabilizing selection for a specific optimal wing-length; and secondly, since size-independent parasitism/predation is important in dampening the size-fecundity relationship, its impact on body-size evolution cannot be neglected, since it may act in concert with other factors (e.g. time-limitation) to elicit a response.

The effect of parasitism on *A. cardamines* in time-constrained environments is likely similar to that in unconstrained ones in the Cheshire area, since model results are similar when mature egg load is substituted for total egg load (Fig. 4.6). This follows from the proportional allocation of resources to capital breeding (the use of stored energy (in butterflies obtained from the larval host-plant) for reproduction (Stearns, 1992; Jönsson, 1997)), which is constant with body-size, i.e. the percentage of mature eggs does not change significantly with wing-length (Fig. 4.2). Hence any evolutionary change in body-size due to parasitism in the study population would not alter the strategic allocation of eggs to capital and income breeding (the use of energy acquired during the reproductive period (in butterflies from nectaring) for reproduction (Stearns, 1992; Jönsson, 1997)). In contrast, Duggan (1985) reported that the proportion of chorionated eggs increases with body mass in Berkshire *A. cardamines*. This could be an effect of stress, since in Duggan's experiments small females were obtained by restricting the food supply to final instar larvae (whereas the Cheshire specimens were fed *ad lib*). If it is not due to stress (see Boggs and Freeman, 2005), then selection against large size by parasitism in the Berkshire population would increase the relative allocation of eggs to income breeding. It is therefore possible that a trade-off between avoidance of parasitism and increased risk of time-limitation exists in some populations but not in others. Severe disruption of the flight period due to inclement weather is likely rarer in Berkshire (southern England) than in Cheshire (northern England); hence greater dependency on income breeding should be less costly in the former environment than in the latter. If so, this may have impacted the evolution of resource allocation to capital and income breeding among different sized specimens in the two areas.

Evolution of larval resting phase behaviour

A. cardamines larvae usually spend the resting phase before contraction to the pre-pupa exposed on the host-plant. When parasitization rates are high, the duration of this period can strongly affect their fitness (Figs. 4.7, 4.8). This implies that longer resting phases should be selected against in environments with high attack rates. Conversely, if short resting phases are physiologically costly, they should be selected against in environments with low attack rates. Hence, the average resting phase duration is predicted to be negatively correlated with attack rate among *A. cardamines* populations. Within populations, fluctuating attack rates could help maintain the variance associated with this trait; the magnitude of the variance would then be positively correlated with the amplitude of the fluctuations.

The existence of two sharply contrasting behavioural phenotypes at the cessation of growth, whereby larvae either vacate the host-plant immediately or remain upon it for the resting phase, is likely an evolutionary response to parasitism. In the absence of physiological constraints, the relative gain in fitness associated with vacating the host-plant may be significant even when attack rates are low (Table 4.5). Importantly, since fecundity does not enter into equation (4.17) for relative fitness change, these results apply equally to males and females; in the absence of constraints, selection should eliminate genotypes which remain on the host-plant even in populations with low rates of parasitism. Since this has not happened in the study area, significant risks are likely associated with early vacation of the host-plant. Although larvae usually cease feeding at the onset of the resting phase, a nutritional threshold may be required to achieve metamorphosis; by remaining on the host-plant, larvae can boost their energy levels if required. In support of this hypothesis, some larvae are observed to increase slightly in size during this period (*pers. obs.*). Hence, those vacating the host-plant early likely risk undernourishment. In environments with fluctuating attack rates, the relative fitness of behaviours associated with avoidance of infection or undernourishment would be constantly changing. This could lead to the evolution of phenotypic plasticity if a cue is available which enables larvae to predict parasitization risk (Benard 2004); inter-seasonal changes in the frequency of the two behavioural phenotypes should then correlate with changes in parasitoid abundance. In the study area the frequency of the two types changed significantly in 2013 (Fig. 4.4c,d), providing circumstantial

evidence for the existence of a cue; however, I have no information on parasitoid abundance that year.

Impact of host use

The models predict that the best host-plant for avoiding parasitism is the large ecotype of *Cardamine pratensis*. The large fitness gains associated with it (Fig. 4.9), which apply to both sexes, imply that in the absence of counterbalancing selection pressures monophagy on this host should be favoured. Interestingly, female *A. cardamines* do show a strong oviposition preference for large *C. pratensis*. However, in the study area it is restricted to a single small field; if it is generally scarce, specialization upon it will be opposed. Moreover, if the parasitic flies happen to find larvae more easily or attack them preferentially on large *C. pratensis*, then its potential advantages as a host will decrease. For the sub-population of larvae which vacate the host-plant before the resting phase, small and large *C. pratensis* are predicted to be better hosts than *Alliaria petiolata*. The combination of these two traits can lead to very large fitness gains for both males and females (Fig. 4.10). The maintenance of the resting phase and oviposition on *A. petiolata* implies the existence of strong counterbalancing selection pressures, of which unequal attack rates, the risk of undernourishment associated with vacating the host-plant early, the scarcity of large *C. pratensis* and the depression of fecundity (due to reduced body-size) on small *C. pratensis* may be important.

The underlying mechanism by which larvae are predicted to avoid parasitism on *Cardamine* is rapid growth (since K_L and K are negative they reduce the values of T or M in equations 4.18, 4.19, 4.21). This exemplifies the slow-growth high-mortality (SGHM) hypothesis (Clancy and Price 1987), whereby herbivores are at higher risk of predation/parasitism on host-plants which prolong growth and hence increase exposure to attack. Very little empirical support for this idea has been forthcoming in relation to parasitoid attack rates on insect larvae growing on different host-plant species (Farkas and Singer, 2013). On the contrary, (externally feeding) larvae tend to be more heavily parasitized on hosts which support rapid growth (Williams, 1999); the SGHM hypothesis only holds among larvae growing on the same host (Benrey and Denno, 1997). This has been interpreted as evidence that parasitoids have evolved a preference for the most nutritious host larvae, which are assumed to

be the fastest growing (Williams, 1999; see also Farkas and Singer (2013) and references therein). However, it is also possible that rapid larval growth has evolved in response to high parasitoid attack rates on specific host-plants. Hence, the failure of the SGHM hypothesis may be more correctly attributed to an escalating antagonistic co-evolutionary interaction between the preference of parasites and the growth rate of their host larvae, in which the most vulnerable slow-growing larvae are selected to grow faster, and in turn parasitoids are selected to preferentially attack fast-growing larvae.

Conclusions

High rates of predation/parasitism have the potential to counteract the size-fecundity relationship when attack rates are size-dependent and so oppose the runaway selection of ever larger sized insects. Even when attack rates are size-independent, predation/parasitism may act in concert with other factors to constrain body-size evolution. In both cases, weak optimality combined with fluctuating attack rates may be important in maintaining the size variance within populations. In *A. cardamines*, parasitism has likely influenced the evolution of larval resting phase behaviour and may have impacted female oviposition preference. These results confirm that spatial variation in attack rates have the potential to generate coevolutionary hotspots and coldspots in the interaction between *A. cardamines* and *P. vulgaris*, resulting in the formation of geographical selection mosaics (Thompson, 2005). Such mosaics have been reported in other host-parasite systems (e.g. Lorenzi et al., 2011).

Chapter 5

Evolution of flowering phenology in *Cardamine pratensis* in response to pre-dispersal seed predation by *Anthocharis cardamines*

Abstract

Phenotypic selection on flowering time by pre-dispersal seed predators can be measurably strong, but the evolutionary outcome of such effects have not been analysed. I here exploit the circumstance that two morphologically distinct ecotypes ("small" and "large") of the brassicaceous herb Cardamine pratensis occurring in the same locality in northern England exhibit different flowering times, which modifies their phenological interaction with the pierid butterfly Anthocharis cardamines. Specifically, I investigate whether the phenology of the C. pratensis ecotypes could have evolved in response to the selection pressure imposed by A. cardamines larvae, which are pre-dispersal seed predators. Over a four year study period, late instar larvae were found to strongly depress plant fitness. The egg-laying and flowering curves for small C. pratensis were strongly peaked and asynchronous, with the primary flowering peak preceding the egg-laying peak by 10-15 days. In addition, an inverse relationship between plant size and flowering time meant that only the smallest plants flowered at the time of peak egg-laying. Since female A. cardamines prefer large, newly flowering plants for oviposition, it is likely that the phenology of small C. pratensis has been modified by the seasonal pattern of herbivory. In contrast, the egg-laying and flowering curves for large C. pratensis were synchronized and egg-loading was 10 times higher than for small C. pratensis. The broad, unpeaked shape of the large C. pratensis flowering curve does however reduce the chances that individual plants will be oviposited on, and may therefore also have been modified by the butterfly. The flowering phenology of Alliaria petiolata (Brassicaceae) has not been modified by A. cardamines, since late instar larvae do not depress fitness.

Introduction

The evolutionary response of flowering phenology to biotic agents remains problematic (Ollerton and Lack, 1992; Elzinga et al., 2007; Kolb et al., 2007; Ehrlén, 2015). If flowering phenology is not under strong selection, phenological variation in plant populations could be due to chance (Ollerton and Lack, 1992). On the other hand, the response of plants to abiotic factors imposing ‘bottom-up’ selection on phenology can be rapid (Elzinga et al., 2007; Franks et al., 2007; Chuine, 2010; Colautti and Barrett, 2013). The ‘top-down’ pressures imposed by biotic factors must therefore be integrated within the context of the whole life cycle (Elzinga et al., 2007; Austen and Weis, 2015; Ehrlén, 2015). Such factors may act either mutualistically or antagonistically; in particular, complex trade-offs may arise

between responses to pollinators and pre-dispersal seed predators (Elzinga et al., 2007; Ehrlén, 2015).

In general, the results obtained over the last two decades support the idea that flowering phenology is subject to strong selection pressures (Munguia-Rosas et al., 2011). However, there is uncertainty over the relative importance of abiotic factors, mutualists and antagonists. In a recent meta-analysis, Munguia-Rosas et al. (2011) concluded that environmental factors associated with latitude were more likely to influence flowering time than pollinators or pre-dispersal seed predators. However, Elzinga et al. (2007) point out that selection pressures imposed by biotic factors are often measurable (e.g. Pilson, 2000), and consider the evidence for the role of biotic interactions in shaping selection on flowering phenology to be strong. Among biotic agents, Ehrlén (2015) argued that evidence for pollinator-mediated selection on flowering time was weak, but stronger for antagonist-mediated selection. This is supported by Kolb et al. (2007), who in a review reported selection on flowering phenology by pre-dispersal seed predators in 80% of tested species.

In general, Munguia-Rosas et al. (2011) found that phenotypic selection tends to favour early flowering; Elzinga et al. (2007) concluded that pollinator-mediated selection favours early or peak flowering, whereas pre-dispersal seed predators select for late or off-peak flowering. The key difficulty is that most studies focus on phenotypic selection gradients which are subject to considerable spatio-temporal variability (Elzinga et al., 2007; Kolb et al., 2007). This gives the impression that the strength and direction of selection is unlikely to be consistent enough to produce an evolutionary response. However, the response of some plant populations to selection can be rapid (Franks et al., 2007; Colautti and Barrett, 2013), so spatio-temporal variation in plant-predator interactions could result in dynamically shifting co-evolutionary geographical mosaics (Thompson and Cunningham, 2002; Thompson, 2005; Kolb et al., 2007).

Phenotypic selection on phenology may be difficult to disentangle from selection on plant size if the two traits are correlated (Munguia-Rosas et al., 2011; Ehrlén, 2015). There is a weak trend in the literature for plant size to be negatively correlated with flowering time (Munguia-Rosas et al., 2011). Large perennial plants may flower earlier in a season than smaller ones due to the accumulation of resources in previous

seasons (Forrest and Miller-Rushing, 2010). This has the potential to modify the phenological interaction with pre-dispersal seed predators if they preferentially target plants in a particular size category, or if fitness losses among different sized plants are unequal (Ollerton and Lack, 1998).

In Europe, *Anthocharis cardamines* is a pre-dispersal seed predator (larvae consume seed-pods) generalizing on Brassicaceous hosts (Wiklund and Åhrberg, 1978), although in Britain it is more strongly associated with *Cardamine pratensis* and *Alliaria petiolata* than with other potential hosts (Courtney and Duggan, 1983). The interactions between the butterfly and these two hosts are therefore more likely to result in coevolutionary geographical selection mosaics in Britain than elsewhere. Interestingly, the butterfly is more strongly associated with *A. petiolata* in the southeast and with *C. pratensis* in the northwest of the British mainland (Courtney and Duggan, 1983; Davies and Saccheri, 2013; Chapter 1), a situation which may reflect a broad scale response to climate (Davies and Saccheri, 2015; Chapter 2). Since flowering phenology is also affected by climate (Menzel et al., 2006), any apparent phenological response of the plants to pre-dispersal seed predation by the butterfly must be interpreted within a geographically localised context.

C. pratensis is an aggregate micro-species polyploid complex exhibiting a wide range of morphological variation. In Continental Europe, this variation is associated with ploidy level; in Sweden, tetraploids and octaploids are visually distinguishable (Arvanitis et al., 2008). In Britain, the situation is complicated, since the range of morphological variation exhibited by octaploids overlaps that recorded for continental tetraploids and octaploids (Dale and Elkington, 1974). *A. cardamines* females are selective in their choice of hosts, preferentially ovipositing on plants with large shoot size (Dennis and Hardy, 2006; Arvanitis et al., 2008) or large flower heads (Dempster, 1997). Since *C. pratensis* is perennial, there is the potential for an interaction between plant size and phenology through the acquisition of resources in previous flowering seasons (Forrest and Miller-Rushing, 2010); different ploidy levels may also be associated with different flowering times (Ramsey and Schemske, 2002). This system is therefore well-suited to assessing the potential impact of pre-dispersal seed-predation on the evolution of plant size and flowering phenology.

In a recent study in Sweden, early flowering *C. pratensis* plants were more heavily attacked by egg-laying *A. cardamines* females in two out of the four years in the study period, with no effect of phenology in the other two years; larger plants were preferentially selected for oviposition in all four years (König et al., 2015). The greater vulnerability of earlier flowering plants corroborated previous results from the same locality (Arvanitis et al., 2008); though intermittent, phenotypic selection against early flowering is likely persistent. However, no information is yet available as to whether plant phenology is responding to this selection pressure. Attack rates can also differ between ploidy types in the Swedish *C. pratensis* populations: *A. cardamines* females prefer to oviposit on octaploids, but tetraploids suffer higher levels of attack at the population level due to their greater occurrence in sunny habitats, at least in some years (Arvanitis et al., 2007, 2008; König et al., 2015). The two ploidy levels do not differ in phenology in sympatric populations, but tetraploid populations often flower 1-2 weeks earlier than octaploid ones among sites (Arvanitis et al., 2008; König et al., 2015).

In a broad scale study utilizing data collected over the entire United Kingdom, Phillimore et al. (2012) found no evidence for a coevolutionary interaction between the phenologies of *A. cardamines* and *C. pratensis/A. petiolata*. Instead, all three species exhibited similar phenological plasticity in responding to temperature cues in an overlapping late winter/early spring time window. However, these conclusions are based solely on citizen scientist observations of dates of first appearance/flowering of the butterfly and its host plants, whereas a wider range of phenological traits may be required to detect the signature of a coevolutionary interaction between them.

In this study, I examine evidence for a coevolutionary interaction between *A. cardamines* and *C. pratensis/A. petiolata* by considering how the shape of the flowering curves may have responded to the shape of the egg-laying curve, and vice versa. I exploit the circumstance that in the Dibbinsdale population there are two sharply distinguished ecotypes of *C. pratensis* (Fig. 5.1), which differ both in size and in phenology: a smaller, early flowering form and a larger, late flowering one (hereafter "small" and "large" *C. pratensis*). This enables me to examine the interaction between size and phenology within and between ecotypes and hence disentangle the selection pressures acting on them. Unlike many previous studies, I adduce evidence for the evolutionary impact of pre-dispersal seed predation on

flowering phenology by relating phenotypic selection to its possible outcome in the two *C. pratensis* ecotypes.



Figure 5.1 Small (left) and large (right) ecotypes *Cardamine pratensis* in Dibbinsdale Nature Reserve (the latter with five orange *A. cardamines* eggs below the central calyces; a sixth egg is partly hidden). Photographed by James Davies at Boden's Hey on 22 March 2012 and at the Upper Tip on 21 May 2012, respectively.

Methods

In Dibbinsdale Nature Reserve small *C. pratensis* and *A. petiolata* both occur abundantly along on the banks of Dibbinsdale Brook and in damp places generally; *A. petiolata* is also found in drier places, especially along footpaths; large *C. pratensis* is restricted to a single dry field situated on high ground (the "Upper Tip", see Chapter 1). *A. cardamines* occurs abundantly in the Reserve; its population size is ~300 in most years (Davies and Saccheri, 2015; Chapter 3). I surveyed the flowering and egg-laying patterns for the three hosts over a 4 year period (2011-14). In all, 983 eggs were found on ~3000 hosts; 187 eggs on ~2000 small *C. pratensis* plants, 550 eggs on ~600 large *C. pratensis* plants, and 246 eggs on ~400 *A. petiolata* plants (plant numbers are approximate since they were not individually counted in 2011).

Host-plant transects

To analyze the phenology of the host-plants and the timing of egg-laying, transects on which every plant was kept under observation from first flowering until dehiscence were selected and revisited every 5-7 days. In 2011, several transects in different areas of the Reserve were chosen for each host (except large *C. pratensis*, which only grows in a single area), and the total number of flowering plants and new

eggs were counted and summed over all transects. This allowed detection of a reasonably large number of eggs (156) on the rarely utilized small *C. pratensis* (a permanent mark was made near every egg found so newly laid eggs could be distinguished from older ones), but unlike in subsequent years the larger number of plants surveyed (>2000) left insufficient time to label and track plants individually. The number of newly flowering plants on each visit was therefore derived from the total number by subtracting the number of previously encountered flowering plants which were still within their flowering period (obtained from data collected in other years). In 2012-14, only one transect was followed per host, and plants were individually labelled (labels were durable against rain and flooding if they were sellotaped to the bottom of the stem with the sellotape covering the label and wrapped around itself). The number of buds, flowers, seed-pods, newly laid eggs, old eggs and larvae were recorded for each plant on every visit.

Survival of *A. cardamines* immature stages

Newly laid eggs on each host species were revisited daily until the disappearance of the egg/larva. Plants were revisited for three days after the last sighting of a larva to ensure that it had not been missed. This work was not restricted to plants in the transects, and spanned a longer period (2009-14, excluding 2011).

Results

The flowering and egg-laying curves for each host were broadly consistent over the four year study period (Fig. 5.2). Small *C. pratensis* always flowered earlier than the other two hosts. Its flowering curve was characterized by a large primary peak in early season followed much later by a small secondary peak. A sharp egg-laying peak usually occurred 10-15 days after the primary flowering peak; its absence in 2012 was due to bad weather. In contrast, large *C. pratensis* displayed a much broader flowering curve with an ill-defined peak occurring at different relative times in different years; the egg-laying curve was also broader than for small *C. pratensis* and was not displaced from peak flowering. *A. petiolata* was characterized by a single flowering peak which in some years was very sharp; egg-laying always followed much later.

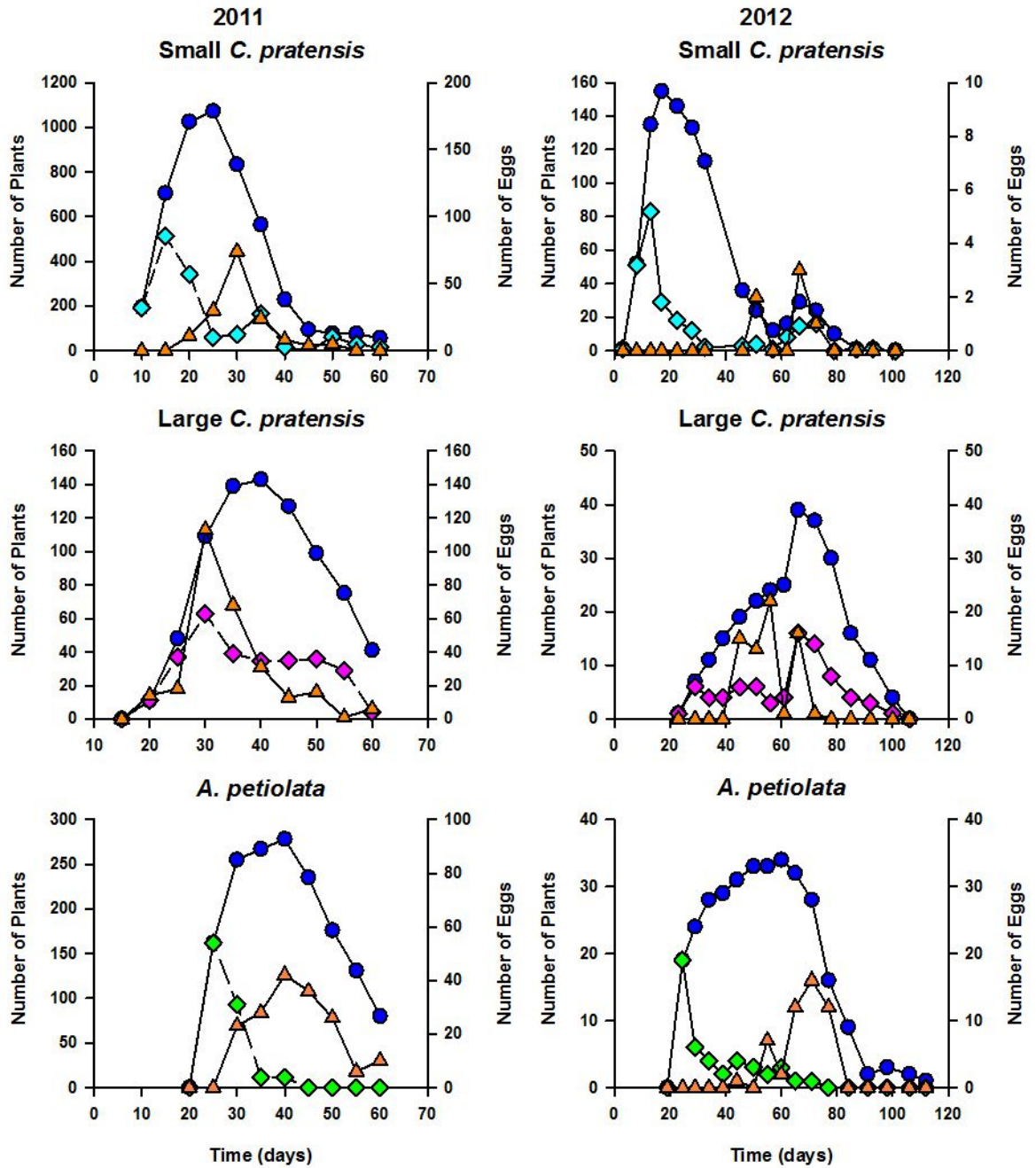


Figure 5.2a Egg-laying and flowering curves for *A. cardamines* and its host-plants in Dibbinsdale in 2011 (left) and 2012 (right). Day zero is the day of first flowering of small *C. pratensis* in the Reserve. In all cases, blue circles show the total number of plants in flower and orange triangles the number of newly laid eggs; diamonds represent the number of newly flowering plants for small *C. pratensis* (cyan), large *C. pratensis* (pink) and *A. petiolata* (green). In 2011, results represent combined data from several transects scattered through the Reserve; in these cases, the number of newly flowering plants are estimates (indicated by dashed lines) derived as described in *Methods*. In 2012, results are for a single transect only and the number of newly flowering plants are accurate counts. Note that in some cases the number of eggs is on a different scale to the number of plants.

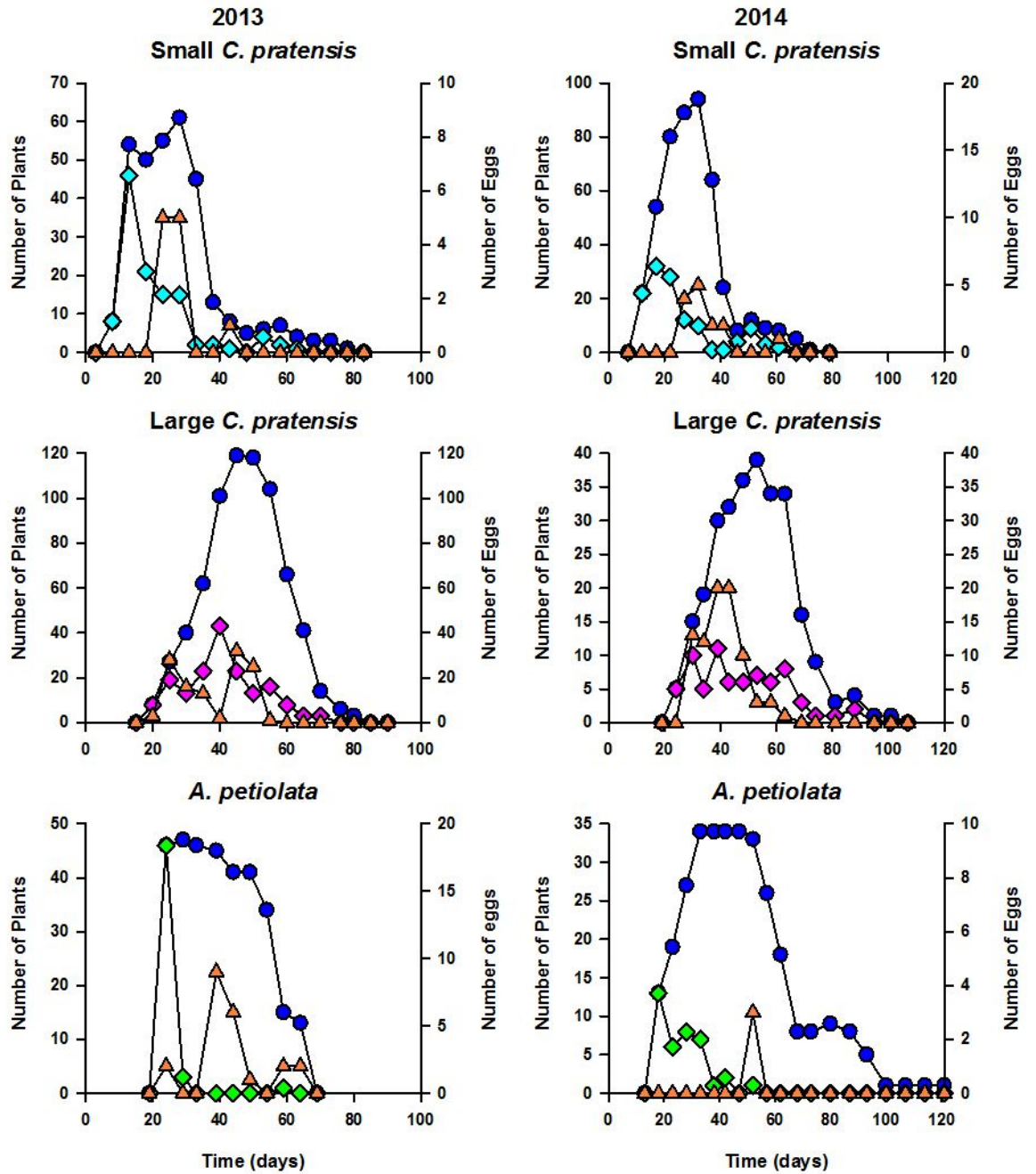


Figure 5.2b Egg-laying and flowering curves for *A. cardamines* and its host-plants in Dibbinsdale in 2013 (left) and 2014 (right). In all cases, blue circles show the total number of plants in flower and orange triangles the number of newly laid eggs; diamonds represent the number of newly flowering plants for small *C. pratensis* (cyan), large *C. pratensis* (pink) and *A. petiolata* (green). In both years values are for a single transect only and the number of newly flowering plants are accurate counts.

These trends are easier to discern when the average flowering and egg-laying curves for the 4-yr period are compared (Fig. 5.3). It is clear that *A. cardamines* egg-laying does not coincide with the initial flowering of the small ecotype of *C. pratensis*, although it is well matched to that of the large ecotype. Furthermore, *A. petiolata* is

not utilized until some considerable time after the commencement of flowering. On the other hand, egg-laying is fairly well matched to the *total* number of plants in flower on the transect for all hosts (Fig. 5.2). Regression analysis on the number of new eggs laid on the transects for the years 2012-14 (data are incomplete for 2011) demonstrates that the number of flowers on the transect had a highly significant effect on the number of eggs laid (Table 5.1); females also strongly favoured large *C. pratensis* over the other two hosts. (The total number of plants is a good surrogate for the number of flowers and is retained in the regression model when the latter are excluded.) This suggests that the visual conspicuousness of the plants *from a distance* is important in determining the number of eggs laid on a transect.

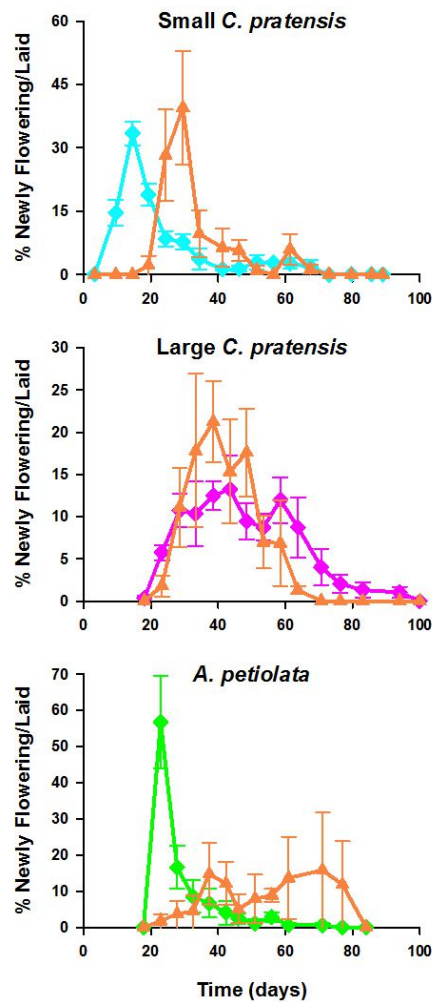


Figure 5.3 Average egg-laying and flowering curves (corrected for differences in plant sample size and weighted by egg loading among years) for *A. cardamines* and its host-plants over the four year study period (2011-2014) in Dibbinsdale (\pm SE for inter-annual variation). The percentage of newly flowering plants are represented by cyan (small *C. pratensis*), pink (large *C. pratensis*) and green (*A. petiolata*) lines; the percentage of newly laid eggs by orange lines. The secondary flowering peak for small *C. pratensis* has been stretched out due to its occurrence at different relative times in different years.

Table 5.1 Regression analysis on the factors affecting the number of eggs laid on a transect. Initially, number of new plants in flower, total number of plants in flower, number of flowers, year and host were entered into the model (the latter as separate dummy variables for each year and host); after backward deletion only the number of flowers and the effect of large *C. pratensis* (L Cp) were retained in the model.

	Unstandardized Coefficients		Standzd Coeffs Beta	t	p	Correlations			Collinearity Statistics	
	B	SE				Zero-order	Partial	Part	Tol	VIF
Const	-.555	.740		-.751	.454					
Flowers	.010	.003	.300	3.856	<.001	.249	.324	.298	.986	1.014
L Cp	5.697	1.031	.429	5.525	<.001	.394	.440	.426	.986	1.014

dependent variable: number of eggs laid on transect.

F = 20.448, $p < 0.001$. Adjusted $R^2 = 0.232$.

At the level of *individual* plants, egg-laying occurred at different times relative to first flowering on the three hosts (Fig. 5.4). Eggs were most rapidly laid on large *C. pratensis* (about 6 days on average after flowering); interestingly, 11.1% of eggs were actually laid on plants *before* first flowering. The average time to egg laying on small *C. pratensis* was over 10 days; however, a sharp mode at 5 days indicates that females prefer newly flowering plants but are either unable to find them efficiently or such plants are too scarce at the time of egg-laying due to their precocious flowering. On *A. petiolata*, eggs were laid about a month on average after first flowering.

Egg-laying was not random with respect to host condition (Table 5.2); female *A. cardamines* tended to select larger plants with a higher number of reproductive units (buds + flowers + seed-pods) to deposit their eggs. This was true for all three hosts and may ultimately have been driven by some combination of plant height (except large *C. pratensis*), number of side-branches, buds and flowers (Table 5.2). Note that the most obvious candidate, the number of flowers, cannot be solely responsible for inducing oviposition since on large *C. pratensis* some eggs were laid before first flowering. Interestingly, the presence of previously laid eggs did not deter oviposition on *A. petiolata* and large *C. pratensis*; conversely, the presence of larvae may have been a deterrent on large *C. pratensis* (Table 5.2).

Table 5.2 Average condition of host-plants utilized or rejected by egg-laying *A. cardamines* females. Utilized plants are weighted by the number of eggs laid on them. Rejected plants are restricted to those flowering at the same time that eggs were laid on other plants. Note that a single plant usually enters into the analysis more than once, since egg laying was assessed repeatedly through the flowering period.

Host	State	N	Ht	Br	Buds	Fl	SP	RU	Old Eggs	Larvae
Ap	-Eggs	353	65.35	2.72	26.54	6.74	22.69	55.97	0.10	0.06
	+Eggs	75	84.13	5.88	45.72	16.71	50.75	113.17	0.56	0.08
S Cp	-Eggs	444	31.79	0.70	4.05	4.59	5.73	14.37	0.05	0.002
	+Eggs	31	35.83	1.29	5.71	7.23	7.81	20.74	0	0
L Cp	-Eggs	773	34.31	0.22	7.00	4.39	4.93	16.32	0.38	0.21
	+Eggs	271	34.83	0.77	15.22	5.05	2.92	23.18	0.62	0.12

Ap - *A. petiolata*; S Cp - small *C. pratensis*; L Cp - large *C. pratensis*; -Eggs - rejected plants (without new eggs); +Eggs - utilized plants (with new eggs); Ht - plant height (cm), Br - number of side-branches; Buds - number of buds, Fl - number of flowers; SP - number of seed-pods; RU - number of reproductive units (buds + flowers + seed-pods); Old Eggs - number of eggs on plant at time of egg laying; Larvae - number of larvae on plant at time of egg-laying.

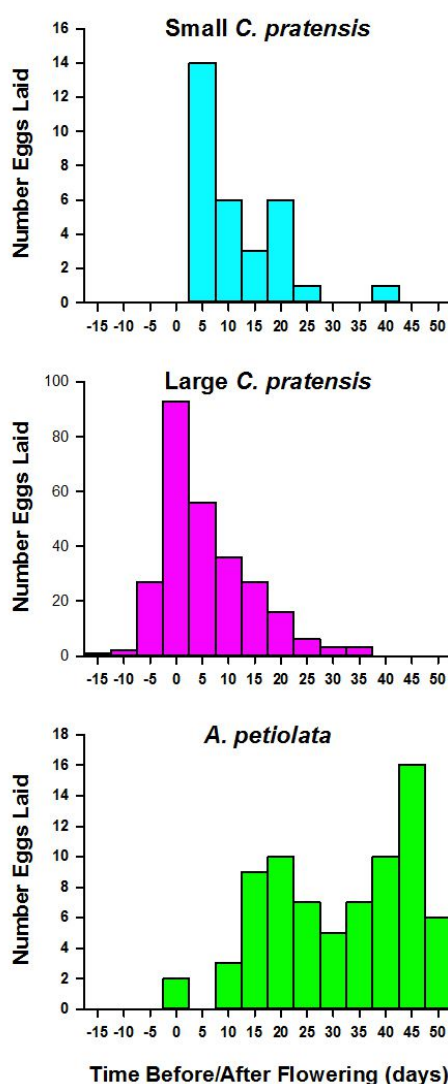


Figure 5.4 Relative timing of *A. cardamines* egg laying in relation to first flowering (day zero) of individual plants of small *C. pratensis* (cyan, mean \pm SE = 11.50 ± 1.44 d, N = 31), large *C. pratensis* (pink, mean = 5.72 ± 0.53 d, N = 270) and *A. petiolata* (green, mean = 31.95 ± 1.58 d, N = 75).

The occurrence of *A. cardamines* eggs on larger hosts did not prevent them from attaining a higher reproductive success, as judged by the final number of mature seed-pods, than smaller plants without eggs (Table 5.3). Interestingly, an important component in the greater success of larger plants with eggs was that a higher proportion of them progressed to dehiscence (%D in Table 5.3); this was especially the case for small *C. pratensis*, although similar trends are observable in the other two hosts. The selection of larger, more successful plants for oviposition by *A. cardamines* females is likely an adaptation to prevent the starvation of late instar larvae, which consume the seed-pods (although such a strategy is unnecessary with respect to *A. petiolata*, it will be generally beneficial). The relative impact of fifth instar larvae on reproductive success differed among hosts. For large *C. pratensis*, the occurrence of mature larvae on larger plants with a higher maximum number of reproductive units depressed the final number of seed-pods to a level lower than that for the smaller plants avoided by egg-laying females (Final SP in Table 5.3); hence the generally higher reproductive success of larger plants selected for oviposition is dependent on larval mortality. For small *C. pratensis*, two of the three plants harbouring fifth instar larvae were wholly consumed and it is clear that reproductive success is severely depressed on this host also (Table 5.3). In contrast, the occurrence of fifth instar larvae on larger *A. petiolata* plants with a higher initial number of reproductive units did not prevent them from gaining a higher level of reproductive success than the smaller ones (Table 5.3).

Table 5.3 The occurrence of *A. cardamines* eggs and fifth instar larvae and the eventual reproductive success of their hosts. (Note that not all fifth instar larvae result from eggs laid on the host, since the caterpillars can crawl between hosts.)

Plant	State	N	Day	Fl Ht	Max Ht	Fl Time	Br	Max RU	Final SP	%M	%D
Ap	-Eggs	91	29.4	39.66	91.37	35.47	4.09	70.62	35.24	49.9	80.2
	+Eggs	42	28.2	40.40	107.94	43.44	6.36	102.74	40.32	39.2	95.2
	5i	15	26.6	38.07	91.23	45.60	7.27	122.93	52.60	42.8	93.3
S Cp	-Eggs	438	21.8	22.64	27.07	18.60	0.79	14.95	3.93	26.3	46.3
	+Eggs	29	23.5	29.07	39.00	24.88	1.66	25.00	12.31	49.2	89.7
	5i	3	34.7	23.33	34.00	20.00	0.67	17.33	4.67	26.9	33.3
L Cp	-Eggs	171	54.6	29.19	34.14	15.94	0.05	11.65	3.11	26.7	44.4
	+Eggs	152	38.6	30.09	41.44	25.63	0.45	20.68	5.75	27.8	53.9
	5i	44	38.1	30.59	42.02	26.95	0.61	22.07	2.07	9.4	40.9

5i - plants with fifth instar larvae; Day - average flowering time of plants; Fl Ht - flowering height; Max. Ht - maximum height; Fl Time - time plants in flower; Max RU - maximum number of reproductive units; Final SP - final number of mature seed-pods; %M - percentage of Max RU which progress to mature SP; %D - percentage of plants progressing to dehiscence. For other abbreviations, see footnote to Table 5.2.

The phenology of the two *Cardamine* ecotypes varied with plant size but in different ways (Fig. 5.5). For small *C. pratensis*, the largest hosts (as measured by the maximum number of reproductive units) flowered first, and there was a sharp decline in the size of the plants over the succeeding flowering periods until a trough was reached about mid-season. Thereafter, larger plants reappeared in the population, although these were not as large as the early season ones. For large *C. pratensis*, there was a more gradual decline in flowering plant size through the season, and there was no late season upturn. Superimposition of the average egg-laying curve onto the phenology-by-size curves for small *C. pratensis* demonstrates that the largest plants, most susceptible to the risk of oviposition, were furthest removed from the time of peak egg-laying, which coincided with the first flowering of the smallest plants least susceptible to the risk of oviposition. In contrast, peak-egg-laying coincided with the first flowering of average sized plants for large *C. pratensis* (Fig. 5.5); there was no obvious temporal avoidance of the egg-laying activity of the butterfly by the more vulnerable plants of this ecotype.

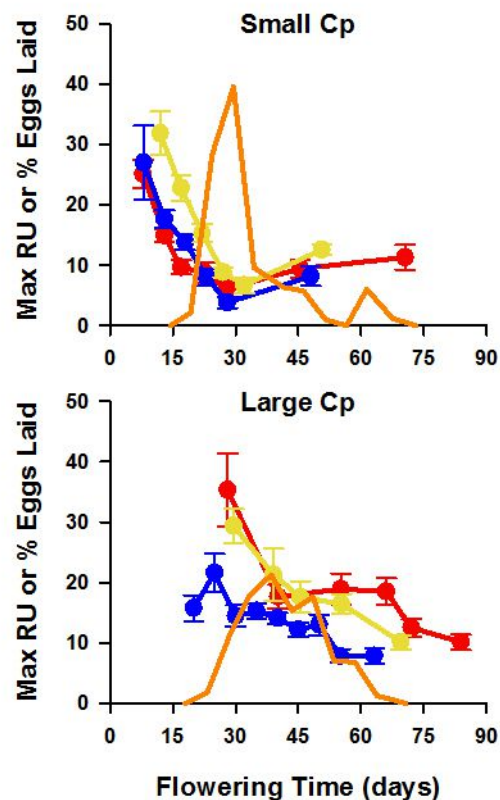


Figure 5.5 Size of small and large *C. pratensis* plants, as measured by maximum number of reproductive units (Max RU \pm SE), against flowering time in 2012 (red), 2013 (blue) and 2014 (yellow). The average *A. cardamines* egg-laying curves are shown in orange.

The *relative* reproductive efficiency of the two *Cardamine* ecotypes (where the intra-seasonal variation in the maximum number of reproductive units is corrected for by assessing the percentage of plants dehiscing or the percentage of reproductive units maturing in successive cohorts) fluctuated with flowering time in all seasons, and the pattern of fluctuation differed between seasons (Fig. 5.6). Hence, the total impact of all biotic and abiotic factors on relative reproductive success was not consistent within and between seasons.

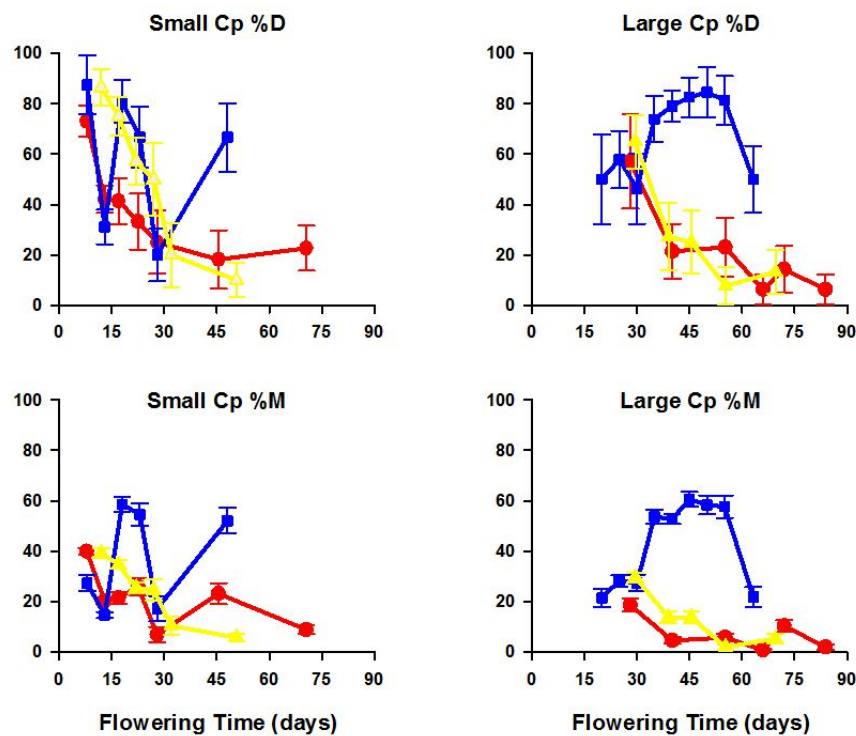


Figure 5.6 Intra and inter-seasonal variation in relative reproductive success (\pm SE) with respect to flowering time for the small and large ecotypes of *C. pratensis*, in 2012 (red circles), 2013 (blue squares) and 2014 (yellow triangles). The measures of reproductive success were the percentage of plants dehiscing (%D) and the percentage of reproductive units reaching maturity (%M).

Egg-loading (the average number of eggs laid per plant) varied among hosts (Fig. 5.7). In general, large *C. pratensis* received most eggs (average value over the 4 yr study period = 0.92/plant), while small *C. pratensis* received the fewest (0.08/plant). This striking difference between the two ecotypes was partly due to phenology (Figs. 5.3 and 5.5) and partly due to the oviposition preference of *A. cardamines* females (Table 5.1). The egg loading on *A. petiolata* was intermediate (0.56/plant), and more susceptible to among-year fluctuations than the other two hosts (Fig 5.7).

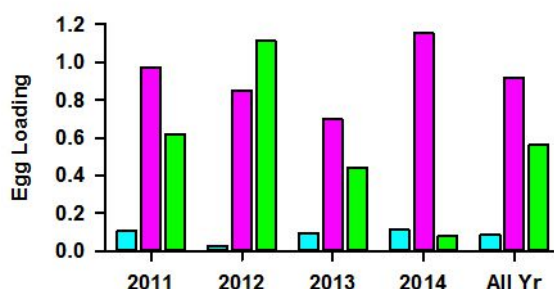


Figure 5.7 Egg loading (number of eggs per plant) on small *C. pratensis* (cyan), large *C. pratensis* (pink) and *A. petiolata* (green) in Dibbinsdale for the years 2011-2014, and the average values over the four years (all years weighted equally).

The survival of *A. cardamines* eggs and larvae varied with host (Fig. 5.8). In five out of the six years surveyed, the immature stages did significantly better on small *C. pratensis* and *A. petiolata*, on which the survival curves were very similar, than they did on large *C. pratensis* (Fig. 5.8). This was due to greater mortality in the egg stage on large *C. pratensis* ($\chi^2 = 24.48$, $df = 1$, $p < 0.000001$ for the comparison of number of eggs surviving versus lost); in consequence, only ~10% of eggs reached the fifth larval instar on this host, whereas ~20% did so on the other two hosts. These patterns were highly consistent among years with one exception in each case. Survival on small *C. pratensis* and *A. petiolata* was very low (~3%) in 2014 (Fig. 5.8), which can be attributed to high losses in the first larval instar ($\chi^2 = 10.01$, $df = 1$, $p < 0.002$ for the comparison of number of first instar larvae surviving versus lost); and survival on large *C. pratensis* was high (~26%) in 2013 (Fig. 5.8), which can be attributed to enhanced survival of eggs ($\chi^2 = 17.92$, $df = 1$, $p < 0.00003$ for the comparison of number of eggs surviving versus lost).

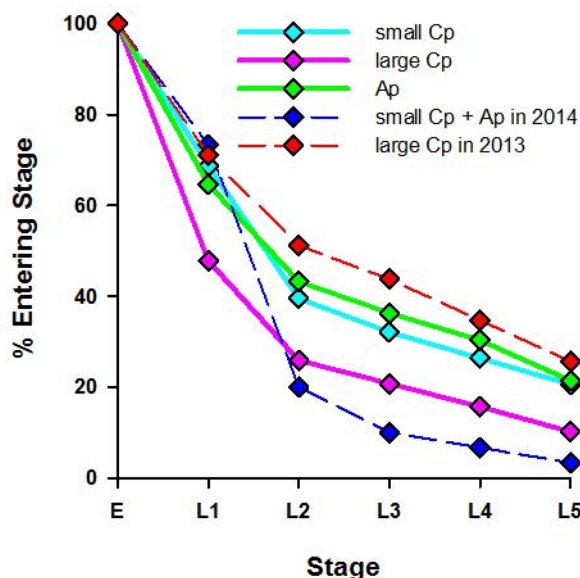


Figure 5.8 *A. cardamines* egg and larval survivorship on different hosts in Dibbinsdale for the years 2009-2014 (excluding 2011). Cp = *Cardamine pratensis*, Ap = *Alliaria petiolata*. Distinct survival curves for small Cp + Ap in 2014 and large Cp in 2013 are plotted separately. Sample sizes: small Cp 243 (excluding 2014), large Cp 255 (excluding 2013), Ap 201 (excluding 2014), small Cp + Ap 32 (in 2014), large Cp 121 (in 2013).

Discussion

Two ecotypes of *Cardamine pratensis* growing in the study locality on the Wirral peninsula in England are characterized by distinct morphologies and phenologies. These contrasting traits are associated with very different susceptibilities to attack by egg-laying *Anthocharis cardamines* females. Specifically, the egg-loading is about 10 times higher on the large, late flowering ecotype than on the small, early flowering one (Fig. 5.7). This raises the question as to whether the morphological and phenological traits of the small ecotype are maintained, and have arisen, at least partly in response to the selection pressure exerted by *A. cardamines*, and if so, why and how the large ecotype is maintained in the population.

The small *C. pratensis* ecotype primarily avoids *A. cardamines* oviposition through phenological escape, since its flowering peak is two weeks earlier than the egg laying peak (Fig. 5.3), and *A. cardamines* females strongly favour newly flowering *C. pratensis* plants (Fig. 5.4; see also Courtney, 1982a). Its smaller size is likely important too, since large plants are more vulnerable to attack than smaller ones (Table 5.3); the much stronger preference of females for the larger ecotype may be related to this (Table 5.1). Chemical oviposition cues may also be involved in the latter response, but I have no information on this subject. In combination, these

morphological and phenological traits result in an average egg-loading of ~ 0.1 for the small *C. pratensis* ecotype (Fig. 5.7). Since about 20% of eggs produce 5th instar larvae (Fig. 5.8), which are largely responsible for depressing the fitness of the plants (Table 5.3; some damage may also be done by 4th instar larvae; prior to this stage, the larvae are not usually harmful), then the fitness of only about $0.1 \times 0.2 = 0.02$ or 1 in 50 plants is depressed by *A. cardamines*. The combination of early flowering and small size is therefore very successful at defending the plants from *A. cardamines* attack.

Alternative explanations for the size and flowering time of small *C. pratensis* are possible. For example, flowering time may be driven by pollination success and the size of the plants may be determined by environmental factors (the large ecotype grows in a different habitat than the small one). Moreover, both ecotypes are perennial and can reproduce vegetatively, and so the force of arguments about the severity of the damage inflicted by 5th instar larvae is lessened by the fact that each plant gets more than one chance to reproduce sexually and can also reproduce asexually.

I believe the balance of evidence favours a herbivore-avoidance interpretation of at least the phenological traits of small *C. pratensis*. The preference of egg-laying females for larger plants implies that the selection pressure exerted by late instar larvae (Table 5.3) varies among size phenotypes. The fact that larger plants maintain a higher reproductive success than smaller ones (Table 5.2) does not mean that they will not respond to the more intense selection pressure imposed on them, since all size phenotypes will be selected to maximize their fitness. Superimposition of the egg-laying curve onto the phenology-by-size curve for small *C. pratensis* (Fig. 5.5) shows that the flowering time of the largest, most vulnerable plants is furthest removed from the time of peak egg-laying, while that of the smallest, least vulnerable ones coincides with it. This strongly suggests an adaptive response to the differential selection pressure exerted by the butterfly. Against this, it could be argued that the earlier flowering time of large plants reflects the prior accumulation of resources in this perennial herb (Forrest and Miller-Rushing, 2010). However, comparison between the two *Cardamine* ecotypes shows that this trend is more pronounced in small *C. pratensis*: the slope of the phenology-by-size curve in early season is much steeper (Fig. 5.5), indicating that flowering time is more strongly

dependent on size in this host. This suggests that even if there is a natural tendency for larger plants to flower earlier, it has been further modified by selection in small *C. pratensis*. Moreover, in late season this ecotype exhibits a small secondary flowering peak (Fig. 5.2) characterized by the return of larger sized plants (Fig. 5.5). This pattern cannot be explained by the prior accumulation of resources; instead, it is strongly suggestive of the outcome of disruptive selection, whereby large, vulnerable plants have moved away from the time of peak egg-laying in both temporal directions. Since the relative reproductive efficiency of the plants does not exhibit a U-shaped curve with flowering time (Fig. 5.6), there is no evidence for consistent depression of plant fitness in mid-season, as might be expected if the totality of biotic and abiotic factors were unfavourable to them at this time. Hence the specific form of the egg-laying curve is the only explanation available for the avoidance of flowering in mid-season.

The phenological escape of small *C. pratensis* is assisted by the egg-laying behaviour of *A. cardamines* females. The dependency of the number of eggs laid on a transect on the number of flowers (Table 5.1) indicates that ovipositing females visually locate large patches of flowering plants from a distance. (Similar behaviour has been recorded by Duggan (1985) and Dempster (1997)). By targeting high density patches, egg-laying efficiency will be increased by minimizing the search time for hosts. Hence, selection for early emergence in the butterfly is likely constrained by the ability of females to locate sparsely distributed plants in early season. This would explain how the first flowering plants of the earliest flowering host (small *C. pratensis*) are able to enter the population before the butterfly begins to emerge. Since females prefer to oviposit on newly flowering plants (Fig. 5.4), early flowerers will also minimize subsequent encounters with egg-laying females. Hence, by flowering early at low density, the largest, most vulnerable small *C. pratensis* plants occupy a relatively safe time window which the butterfly is unlikely to invade due to constraints operating on the efficiency of its egg-laying behaviour.

The impaired ability of females to locate sparsely distributed plants in *late* season likely explains both the occurrence and limited extent of the small *C. pratensis* secondary flowering peak at that time. If these late flowering plants were more abundant, their fitness would be depressed through increased egg-laying efficiency; the small size of the secondary peak is therefore likely maintained by frequency-

dependent selection. The larger size of the early flowering peak may also reflect the fact that, of the two strategies, early flowering is likely to be more consistently successful than late flowering, since once the butterfly has emerged it will be an ever present danger, particularly in seasons when bad weather pushes back its flight period. This is confirmed by my data in 2012 (Fig. 5.2), when the usual egg-laying peak two weeks after peak flowering was absent due to the intervention of bad weather. However, eggs did subsequently appear as usual in late season, confirming the greater vulnerability of plants flowering at this time.

Support for a herbivore-avoidance mechanism is provided by a simple mathematical model (Appendix 10), which demonstrates that if the fitness of plants varies inversely with plant size and with the flower density at any particular time on a transect (due to the egg-laying activity of female butterflies), and if the fitness cost in the early/late flowering periods is reduced due to the greater scarcity of the butterfly in those periods (more so in early season), then the evolutionarily stable flowering curve is bimodal (Fig. A10.1c in Appendix 10), and the average size of the plants is largest in early season, smallest in mid season and intermediate in late season. The latter effect is due to the stronger selection pressure exerted on larger plants by egg-laying females, which pushes them into the safer early/late flowering periods more quickly; once there, they prevent the invasion of the smaller plants due to the cost of increased flower density. These results are in good agreement with the actual phenology-by-size curve for small *C. pratensis* (Fig. 5.5; compare Fig. A10.2 in Appendix 10).

The large ecotype of *C. pratensis* is subjected to intense attack by *A. cardamines*, with an average egg-loading of ~ 0.9 (Fig. 5.7). Egg to 5th larval instar survivorship is usually $\sim 10\%$ (Fig. 5.8), so about 9% of plants are predicted to incur severe damage in most years. In spite of this strong ongoing selection pressure, the plants have not responded in the same way as the small ecotype. It may be that among ecotypes large size and late flowering are tightly coupled (though clearly not so within ecotypes), so that early flowering is not possible in large *C. pratensis*. This would mean there is no accessible early season time window when the butterfly is rare or absent that the plants could invade. However, it is possible that the broad, unpeaked form of the flowering curve (Fig. 5.3) is a response to the butterfly. Since the number of eggs laid on a transect is dependent on the number of flowers, and

since this effect is stronger for large *C. pratensis* than for the other two hosts (Table 5.1), it follows that plants flowering at times when few other plants are in flower will be selected. A mathematical model (Appendix 10) in which the fitness cost associated with flower density is constant through the season shows that the evolutionarily stable flowering curve is broad, with an approximately equal number of plants flowering in each time period (Fig. A10.1b in Appendix 10). While not as successful as a phenological shift, such a response could be critical in boosting the average reproductive success to a sustainable level. Moreover, the rate of egg to 5th larval instar survival is usually half that occurring in the other hosts (Fig. 5.8), which may also be important (in its absence, 18% of plants would be severely damaged). This difference is attributable high egg mortality, which demonstrates that low survivorship is due to high predation, rather than to the condition (e.g. toxicity) of the plants (which would only affect larval survivorship; cf. Courtney, 1981). It is possible that large *C. pratensis* can only establish itself in habitats where the number of *A. cardamines* egg/larval predators is high.

In contrast with the two *Cardamine* hosts, the reproductive success of *Alliaria petiolata* is not depressed by fifth instar *A. cardamines* larvae. On average, there are over 100 reproductive units (buds/flowers/seed-pods) on the larger specimens of this host selected for egg-laying (Table 5.3), so that the loss of a small number of them due to larval grazing is not significant. The scarcity of eggs on plants at and shortly after first flowering cannot therefore be attributed to a phenological shift; it most likely reflects the inconspicuous appearance of the plants before they are in full flower, and perhaps the butterfly's preference for the *Cardamine* species (note the late season surge in egg-laying (Fig. 5.3) when *Cardamine* is in decline).

In conclusion, it is likely that the early flowering time of small *C. pratensis* and the broad flowering curve of large *C. pratensis* represent adaptive responses to prevent/minimize egg-laying by female *A. cardamines*. The former host is very successful at avoiding egg-laying, whereas the latter is best viewed as "making the best of a bad job" (Maynard Smith, 1982). This suggests that the two ecotypes are in different phases of a coevolutionary interaction with the butterfly. The question remains as to how localized these responses are: whether the same ecotypes in different populations exhibit the same traits, or whether there are hotspots and coldspots in the coevolutionary interaction with *A. cardamines* leading to different

trait values among populations, as predicted by the geographical mosaic theory of coevolution (Thompson, 2005). The phenology of *C. pratensis* was on average 19 days earlier than that of *A. cardamines* in England and Wales for the period 1883-1947 (Sparks and Yates, 1997), but unfortunately records for *C. pratensis* during this period are scarce (15 years only). Interestingly, Dempster (1997) found a strongly negative relationship between plant size and flowering time for *C. pratensis* growing in Monks Wood, Huntingdonshire (south England), where *A. cardamines* is also abundant. The interaction with egg-laying phenology was not investigated, but Duggan (1985) observes that "in woodland glades...[*C. pratensis*]...normally escapes butterfly attack by virtue of early flowering". In Monks Wood, larger plants did not reappear in late season (Dempster, 1997), so selection in this locality (if occurring) has been directional rather than disruptive (as in Dibbinsdale). On the other hand, *C. pratensis* was late flowering at Alderley Edge, Cheshire, in 2005 (Dennis and Hardy, 2006), and egg-loading was much higher than in the Dibbinsdale populations (1.375). In Sweden, Arvanitis et al. (2008) report that "the latest flowering [*C. pratensis*] individuals [both tetraploids and octaploids] completely escaped [*A. cardamines*] seed predation"; however, their Fig. 2 suggests that bidirectional selection for early and late flowering may have occurred in Sweden also. On the islands of Ljustero and Ingarö, *C. pratensis* flowers after *A. cardamines* females emerge (Wiklund and Åhrberg, 1978; Wiklund and Friberg, 2009); on Ljustero, egg loading is fairly high (0.43 eggs/plant) but accompanied by very low egg + larval survivorship (4.5%), due to a high loss rate (70%) of first instar larvae (Wiklund and Friberg, 2009). Hence the same traits associated with defence against *A. cardamines* in the Dibbinsdale population reappear in different combinations in different populations in Britain and Sweden. The evidence therefore favours the idea that geographical selection mosaics have formed in response to the coevolutionary interaction between *C. pratensis* and *A. cardamines* among localities.

Chapter 6

Evolution of adaptive phenotypic plasticity and advancement in phenology of *Anthocharis cardamines* with respect to its host-plant *Cardamine pratensis*

Abstract

Adaptive phenotypic plasticity is predicted to evolve in preference to local genetic differentiation given the existence of reliable cues. In insects, host-plant utilization and climate change can give rise to plastic responses. Males of the pierid butterfly Anthocharis cardamines are smaller and emerge earlier when utilizing the Brassicaceous host-plant Cardamine pratensis. I here examine whether early emergence could have evolved in response to selection on small phenotypes initially produced by undernourishment. By rearing larvae on a restricted diet, it was possible to disrupt the developmental path leading to early emergence, suggesting that the ancestral response to food limitation was likely different to the stress-tolerant response observed today. The adaptive hypothesis is further supported by the depressed dispersal of small imagines, which is predicted to select for early emergence. A. cardamines is currently advancing phenologically, and has become a key indicator species for climate change. In a local population in northern England, advancing phenology is associated with depression of wing-length. I show that this is likely due to small, early emerging females being selected to oviposit on C. pratensis, which currently flowers considerably in advance of the appearance of the butterfly. This cautions against interpreting the long-term phenological response of this species solely in terms of an unaltered plastic response to climate change, although global warming will likely be critical in allowing A. cardamines to invade C. pratensis' phenological space. The butterfly's responses to host-plant and climate change support current ideas that pre-existing phenotypic plasticity in developmental processes can act as a starting point for adaptive evolutionary change.

Introduction

The theory of geographic selection mosaics assumes spatial and temporal variation in coevolutionary interactions between organisms, leading to genetic differentiation among populations (Thompson, 2005). However, phenotypic plasticity has the potential to track changes across landscapes and through time, allowing organisms to respond quickly to their immediate environment. For this reason, adaptive phenotypic plasticity is predicted to evolve in environments with high spatio-temporal heterogeneity, given the existence of reliable cues (Scheiner, 2013). A high dispersal rate between demes also favours plastic responses over genetic differentiation, at least when selection acts before dispersal, i.e. when selection takes place in the same environment in which the organism is phenotypically determined (Schiener and Holt, 2012; Scheiner, Barfield and Holt, 2012). Yet the impact of

phenotypic plasticity on the coevolutionary process has only recently begun to be explored (Scheiner, Gomulkiewicz and Holt, 2015). Preliminary results suggest that the coevolutionary process is little affected by whether the traits involved in interactions are plastically or genetically determined; conversely, the evolution of plasticity is predicted to be affected by coevolutionary interactions, particularly when antagonistic (Scheiner, Gomulkiewicz and Holt, 2015).

In spite of the apparent advantages associated with phenotypic plasticity, local adaptation is common (Hereford, 2009). This suggests that phenotypic plasticity may be relatively unimportant in the formation of selection mosaics. However, the plastic response to host-plant utilization in male *A. cardamines* is likely an adaptation to the habitat characteristics associated with those host-plants, and hence important in regulating the coevolutionary response at the landscape level (Davies and Saccheri, 2013; Chapter 2). The "emerge early and wait" mate finding strategy resulting from *C. pratensis* utilization will be advantageous in high density core populations, while the "emerge late and rove" strategy resulting from *A. petiolata* utilization will be beneficial in open, low density ones (Davies and Saccheri, 2013; Chapter 2). Such plasticity adapts males to whichever environment they developed in, with the exception of those in which both host-plants occur together. In this case, a stay-or-go response (itself likely plastic) redistributes late emerging males to areas of the landscape less populated with early emerging competitors (Davies and Saccheri, 2015; Chapter 3). The evolution of plasticity in this case is consistent with a high rate of interchange between *A. cardamines* demes utilizing *C. pratensis* and *A. petiolata* (Schiener and Holt, 2012; Scheiner, Barfield and Holt, 2012), which would oppose local differentiation.

An important question regarding adaptive phenotypic plasticity is to what extent it arose through inherent sensitivity of the developmental process to environmental variation, or was moulded into its present form by natural selection (West-Eberhard, 2003; Ghalambor et al., 2007). In this respect, the association between small size and early emergence in male *A. cardamines* is interesting, since with butterflies it is more usual for large specimens to emerge before small ones (M. Singer, *pers. com.*; Carvalho, Queiroz and Ruzsczyk, 1998). Davies and Saccheri (2013) assumed that the initial plastic response to *C. pratensis* was depressed size due to nutritional deprivation, and that this was obligately coupled with depressed dispersal; early

emergence was then selected in high density populations in response to the latter trait (Davies and Saccheri, 2013; Appendix 2). Hence, of the three plastic traits associated with *C. pratensis* utilization, two (small size and depressed dispersal) are hypothesized to have arisen by chance, and the third (early emergence) to have evolved through natural selection.

I here test two predictions relating to this hypothesis. First, I examine whether inter-deme variation in *C. pratensis* plant-height (as an index of nutritional quantity) is positively associated with *A. cardamines* larval body-length. This tests the null hypothesis that small size on this host-plant is *not* due to nutritional limitation, in which case the phenotypic size should be unresponsive to fluctuations in nutritional quantity. Second, I compare the effects of unrestricted and restricted diets on the emergence timing of *A. cardamines* imagines. If the restricted diet treatment reduces nutrition below a threshold of starvation, it will disrupt the normal developmental response and reveal whether precocious emergence timing is dependent on it. This will provide a strong indication of whether the assumed ancestral response to nutritional deprivation has been modified by selection.

Phenotypic plasticity can only be selected to adapt organisms to the range of environments they commonly experience. This means that plastic traits evolved in response to past environments may be insufficiently adapted to cope with changes resulting from habitat destruction and climate change. In insects, emergence timing frequently responds plastically to thermal cues (e.g. Tauber and Tauber, 1976; Valtonen *et al.*, 2011; Posledovitch *et al.*, 2014), enabling species to accurately track inter-annual variations in seasonal temperatures. Insofar as this trait is adapted to the average temperature profile of local environments, climate change may render existing adaptive responses maladaptive. In particular, if the response to altered temperature profiles differs between insect herbivores and their host-plants, it will lead to phenological mismatching between the two trophic levels (e.g. Visser and Holleman, 2001; van der Putten *et al.*, 2004; Gilman *et al.*, 2010). This would impact the ongoing coevolutionary interaction between them (Singer and McBride, 2012).

In spring flying moths which overwinter as pupae, phenology is most frequently controlled by a combination of temperature and photoperiod (Valtonen *et al.*, 2011), the latter cue presumably acting to prevent unseasonably early emergence due to

warm spells in winter. Since *A. cardamines* overwinters in the pupal stage, one might expect its emergence timing to be controlled by a similar mechanism, but so far only temperature has been implicated in it (Sparks and Yates, 1997; Posledovitch *et al.*, 2014; Stalhandske *et al.*, 2014); premature emergence is prevented by the more risky strategy of a cold duration requirement (Posledovitch *et al.*, 2015a; Stalhandske *et al.*, 2015). This is consistent with the fact that this species is showing a marked response to climate change (Roy and Sparks, 2000; Brooks *et al.*, 2014; Karlsson, 2014). Hence, there is the potential for phenological mismatching between *A. cardamines* and its host-plants (Posledovitch *et al.*, 2015b); however, the generalist host utilization strategy of *A. cardamines* in Sweden may be resilient to such changes (Navarro-Cano *et al.*, 2015).

In the context of geographical selection mosaics, phenological response to climate change could have the *opposite* effect to phenological mismatching, since phenologies may already be mismatched between antagonistic organisms, if the prey/host species currently has the evolutionary advantage over the predator/parasite/herbivore species. This possibility clearly applies to the *A. cardamines* population in Dibbinsdale, and perhaps more generally to orange-tips in Britain, since *C. pratensis* flowers well in advance of the emergence of females (Chapter 5). Since the emergence time of *A. cardamines* is advancing, its phenological relationship with its host-plants could be changing. In Britain, the response of the butterfly to temperature appears to be in step with that of its host-plant *A. petiolata* (Sparks and Yates, 1997; Harrington, Woiwod and Sparks, 1999), but its relationship with *C. pratensis* is less certain (Sparks and Yates, 1997). Moreover, if females are responding to the selection pressure exerted by early flowering *C. pratensis*, then an advancement in phenology may not be due to climate change at all, although it could be easily mistaken for it. Interestingly, models of *A. cardamines* emergence timing in Britain suggest that the long-term advancement in its phenology is partly independent of temperature (Roy and Sparks, 2000; Brooks *et al.*, 2014). Given the importance of *A. cardamines* as an indicator species for climate change, it is important to know whether genetic, as well as phenotypically plastic, effects are contributing to its advancing phenology.

In Dibbinsdale, *A. cardamines* phenology has been advancing and its average wing-length has been decreasing since monitoring of the population began in 2003 (Figs.

6.1 and 6.2). I here investigate whether these effects are linked, and whether they involve genetic or phenotypically plastic changes. I obtain estimates for the heritabilities of wing-length and emergence timing, and the genetic correlations between them (cross-trait and cross-sex), to elucidate whether the observed phenotypic changes in Dibbinsdale could be a response to selection. In particular, the correlated responses will help determine whether the changes are best accounted for by selection operating on both traits in both sexes, or whether the response of one of the traits/sexes is an indirect effect resulting from its genetic correlation with the other trait/sex. These data will also be valuable in assessing the likelihood of the finely-tuned adaptively plastic responses associated with these traits discussed in previous Chapters. More generally, elucidation of the genotype by environment interactions associated with host utilization should help clarify how the coevolutionary interaction between *A. cardamines* and its host-plants is evolving. This in turn will assist interpretation of future phenological changes involving these key indicator species in relation to climate change.

Methods

To investigate inter-deme variation in *C. pratensis* plant-height, specific transects were selected at each sub-site in Dibbinsdale Nature Reserve along which all plants were measured. Each ramet was measured to the nearest cm at the time of flowering, and marked with permanent marker pen to prevent resampling. Each transect was visited every few days until an adequate number of ramets had been sampled. Since mean plant-height varied continuously between sub-sites, the distinction between the "small" and "large" ecotypes was weakened, although the "large" ecotype did produce the tallest plants. For the purposes of analysis, the ecotype distinction was therefore dropped (for reference, the "large" ecotype refers to the population growing at the Upper Tip).

A. cardamines larval body-length and adult wing-length measurements were obtained as described in Chapter 2.

The developmental response of *A. cardamines* to a restricted diet was analysed for individuals of the same family, to minimize genetic variance in the traits of interest (wing-length and eclosion day). All larvae were reared through the 4th instar on *A. petiolata*, then assigned at random to *A. petiolata* and (small) *C. pratensis* in the 5th

(final) instar. A further random sample from both host-plant treatments was then selected for the restricted diet treatment, in which food was withdrawn when larvae reached 25 mm in length. The remaining larvae were reared *ad lib.* on *A. petiolata*. Numbers were too small to allow a full factorial design in which larvae were also reared *ad lib.* on *C. pratensis*; a second family was therefore raised in parallel in which all larvae were raised *ad lib.* on both host-plants.

Phenological records of the first flowering date of *C. pratensis* and the first emergence date of *A. cardamines* in Dibbinsdale were obtained by systematically searching the Reserve every day from about a week before these events were due to occur.

All heritabilities were assessed for individuals reared exclusively on *A. petiolata*, to prevent interference from the phenotypically plastic response associated with host use (Davies and Saccheri, 2013; Chapter 2). Specimens were paired in outdoor breeding cages placed in full sunlight with a flowering host-plant as a nectar source. Since the butterflies could not be watched, the female was assumed to have mated after one day with the male, and on subsequent days was left alone in a cage in full sunlight to oviposit on the host-plant, which also acted as a nectar source. (The insects would not mate or lay eggs indoors, or outdoors without full sunlight.) All larvae were reared under uniform conditions indoors; the resulting pupae were moved to an outhouse for the winter, then transferred to a refrigerator (4C) to prevent premature development in spring. Eclosion began a few weeks after pupae were removed from the fridge. Families contributing to the analyses were raised in three successive years (2011-2013).

Narrow-sense heritabilities (h^2) for wing-length and eclosion day were calculated from the slope of offspring on mid-parent regressions (after correcting for the effects of sex and year). Genetic correlations (r_A) were calculated from:

$$r_A = \frac{\text{cov } XY}{\sqrt{\text{cov } XX \cdot \text{cov } YY}}$$

where covXY is the covariance between parental trait X and offspring trait Y, and covXX and covYY are the cross-covariances between the parental and offspring traits. Since there are two possible estimates of covXY, r_A was taken as the

arithmetic mean of the two correlations resulting from them (Akesson, Bensch and Hasselquist, 2007). The standard errors of these estimates were calculated using the formula in Falconer and Mackay (1996):

$$\sigma_{r_A} = \frac{1-r_A^2}{\sqrt{2}} \sqrt{\frac{\sigma_{h_X^2} \cdot \sigma_{h_Y^2}}{h_X^2 \cdot h_Y^2}}$$

Intersexual genetic correlations were calculated from the Pearson correlation between the family means for each sex.

Results

Longitudinal changes in phenology and body-size

The phenology of *A. cardamines* males on the Wirral peninsula has advanced during the period 1982-2015 (Fig. 6.1), consistent with UK national records. Accurate dates of first appearance for the period 2001-2015 in Dibbinsdale show an advancement of nearly 1 day per year. In parallel with this, the mean wing-length of both males and females in the Dibbinsdale population has decreased by about 0.06 mm per year during the period 2003-2015 (Fig. 6.2).

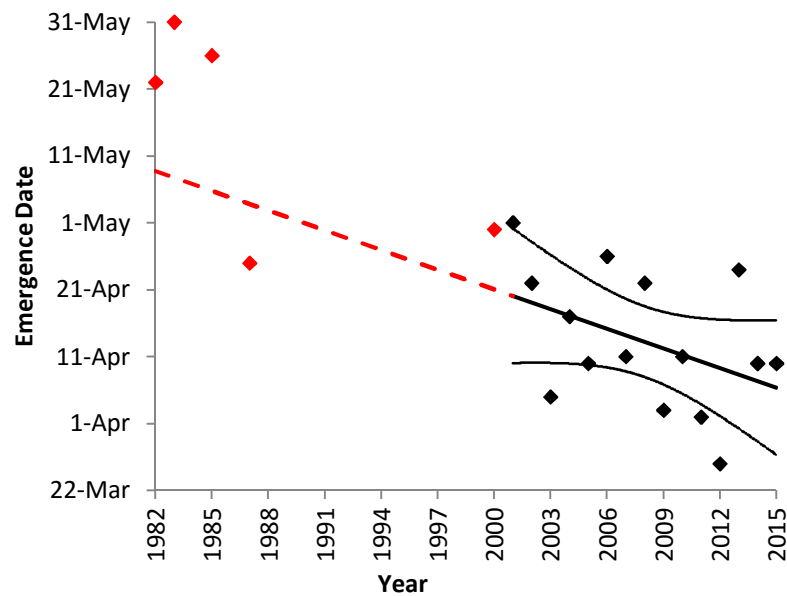


Figure 6.1 First sighting of male *A. cardamines* on the Wirral peninsula by the author for the period 1982-2015. Records for the period 2001-2015 (black symbols) are accurate and were used to derive the best-fit line \pm 95% confidence bands (gradient = -0.98, $R^2 = 0.19$, $p = 0.11$). This has been extrapolated backward (dashed line) through the period 1982-2000 for which records (red symbols) are more casual and intermittent.

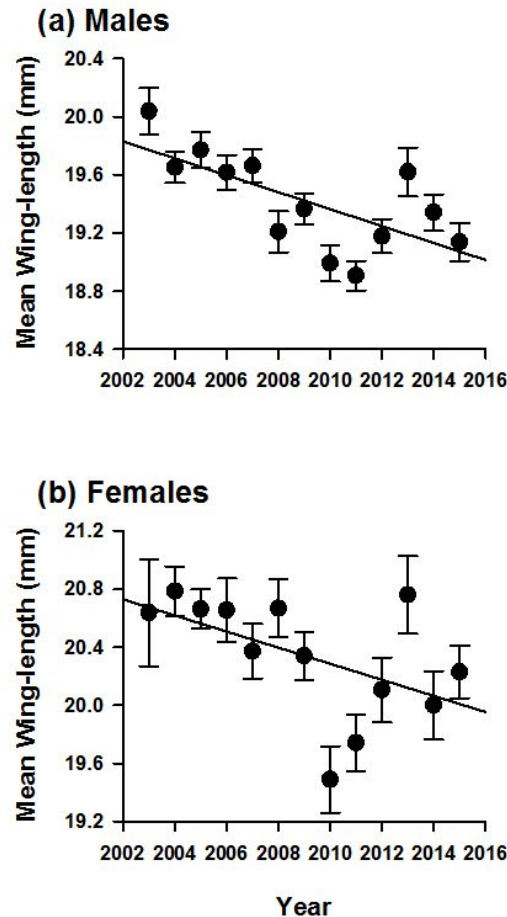


Figure 6.2 Longitudinal changes in mean (\pm SE) male and female *A. cardamines* wing-length in Dibbinsdale Nature Reserve. Regression equations: $WL = -0.058 \cdot Yr + 136.4$, $p = 0.01$, $R^2 = 0.46$ (males); $WL = -0.055 \cdot Yr + 131.4$, $p = 0.07$, $R^2 = 0.27$ (females). [WL = wing-length, Yr = year]

Body-size phenotypic plasticity on *C. pratensis*

Sub-site variation in mean *C. pratensis* plant-height was largely consistent during the period 2007-2011, with plants growing at Otters' Tunnel usually being the shortest, and those growing at the Upper Tip always being the tallest (Fig. 6.3). Across sub-sites, the plants varied in step with each other among years, with the exception of those growing at Spital, where plant-height increased by a disproportionate amount in 2009 and increased rather than decreased in 2010. This strongly suggests that annual variation in plant-height is influenced by the same abiotic factors in all sub-sites.

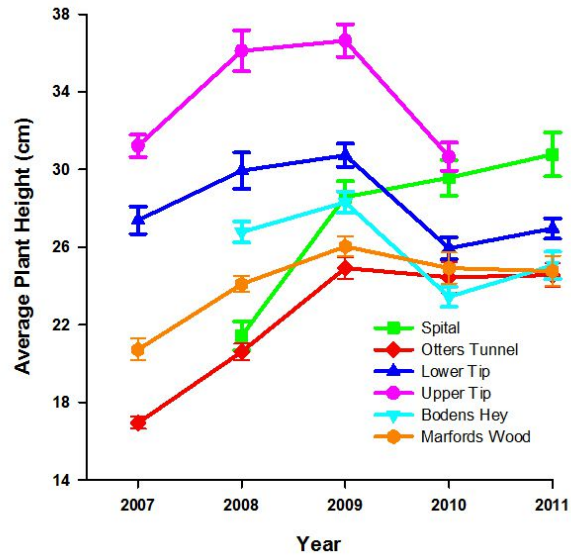


Figure 6.3 Sub-site and yearly variation in *C. pratensis* average plant-height (\pm SE) in Dibbinsdale Nature Reserve for 2007-2011.

Variation in mean sub-site *C. pratensis* plant-height was strongly correlated with variation in *A. cardamines* larval-length (Fig. 6.4). This implies that the average height of plants in the whole sub-site population (i.e. not restricted to those on which larvae developed) is a good predictor of average larval-length (Fig. 6.5), and hence, via the relationship between wing-length and larval-length (Chapter 2), of average imaginal size. This is supported by regression analysis, which demonstrates that average sub-site plant-height is a significant predictor of the following years' average sub-site male wing-length (Table 6.1). Hence, inter-deme variation in plant-height leads to corresponding variation in larval/imaginal body-size, with the clear implication that, on this host, nutrition is a limiting factor. The occurrence of year as a separate effect in the regression model (Table 6.1) demonstrates that the long-term decline in wing-length (Fig. 6.2) is independent of *C. pratensis* plant-height, and hence cannot be attributed to changes in this host-plant's nutritional quality/quantity.

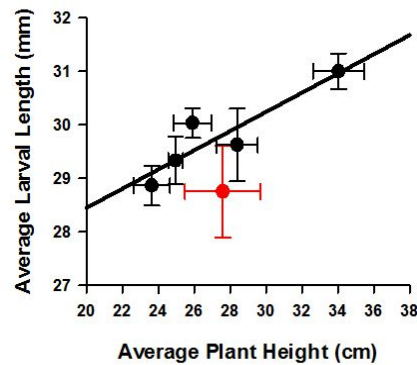


Figure 6.4 Average larval-length (\pm SE) as a function of average *C. pratensis* plant-height (average of the means over a 4 yr period \pm SE) in five sub-sites at Dibbinsdale in 2008-2011 (a sixth sub-site (Spital, shown in red) has been excluded from the regression since only 4 mature larvae were sampled there). Regression equation: $\text{Length} = 0.18 * \text{Height} + 24.86$, $R^2 = 0.83$, $p = 0.03$. The sub-sites are, from left to right (with number of larvae): Otters' Tunnel ($N = 22$), Marford's Wood ($N = 9$), Boden's Hey ($N = 30$), Lower Tip ($N = 8$), Upper Tip ($N = 33$).

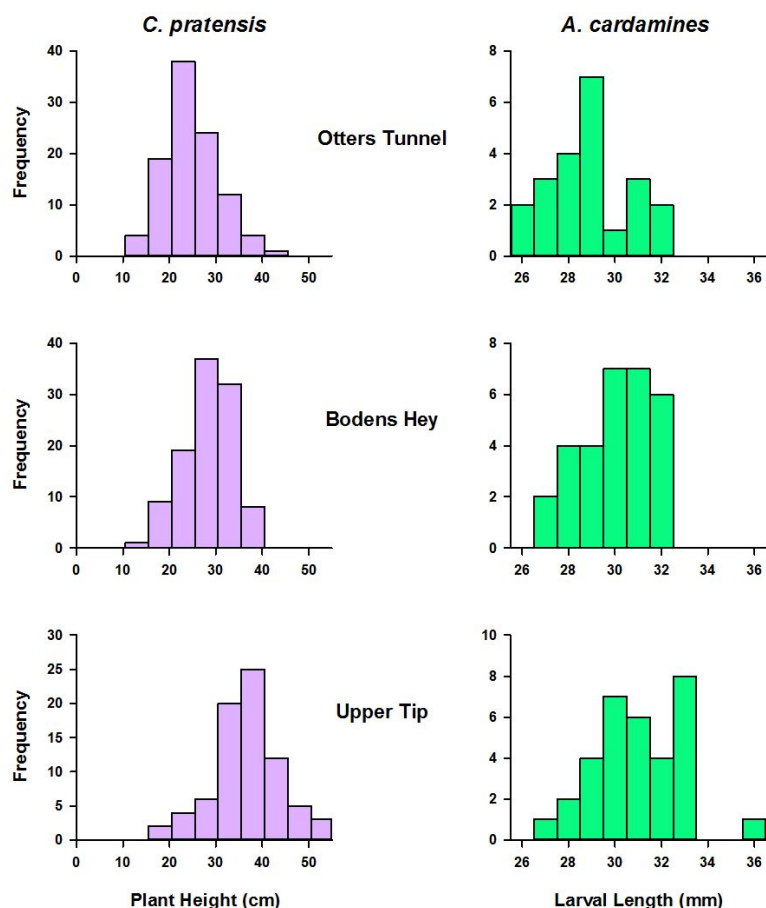


Figure 6.5 *C. pratensis* plant-height (2009) and *A. cardamines* larval-length (2008-11) distributions at the Otters' Tunnel, Boden's Hey and Upper Tip sub-sites in Dibbinsdale. Means \pm SE for *C. pratensis*: 24.92 ± 0.57 mm, $N = 102$ (Otters' Tunnel); 28.31 ± 0.55 mm, $N = 106$ (Boden's Hey); 36.64 ± 0.84 mm, $N = 77$ (Upper Tip); means \pm SE for *A. cardamines*: 28.86 ± 0.37 mm, $N = 22$ (Otters' Tunnel); 30.03 ± 0.28 mm, $N = 30$ (Boden's Hey); 31.00 ± 0.33 mm, $N = 33$ (Upper Tip).

Table 6.1 Effect of year and sub-site average *C. pratensis* plant-height on the following year's average wing-length of male *A. cardamines* at those sub-sites in Dibbinsdale Nature Reserve for the period 2007-2011.

	Unstandardized Coefficients		Standzd Coeffs Beta	t	p	Correlations			Collinearity Statistics	
	B	SE				Zero-order	Partial	Part	Tol	VIF
Const	253.4	124.1		2.042	.055					
Year	-.117	.062	-.369	-1.896	.073	-.281	-.390	-.362	.961	1.041
Height	.046	.020	.446	2.291	.033	.373	.456	.438	.961	1.041

dependent variable: Male Wing-length

F = 3.709, p = 0.043. Adjusted R² = 0.198.

The ontogenetic effects of nutritional deprivation on *A. cardamines* were investigated in a set of experiments in which larvae were removed from the host-plant before full maturation, and hence forced to pupate at small size (Table 6.2). There were two families, the first (A) being a control in which larvae reared on *A. petiolata* through the 4th instar were split between small *C. pratensis* and *A. petiolata* in the fifth (final) instar. The results confirm that males are smaller and emerge earlier when reared on *C. pratensis* (Table 6.2), and demonstrate that the switch controlling this plastic response takes place in the fifth instar. In the second family (B), larvae were split between the two host-plants as in the control, but food was withdrawn from some of them when they reached 25 mm in length (restricted diet). This treatment caused high mortality (6 out of 14 specimens (43%) were unable to pupate); the following conclusions are therefore subject to the caveat that mortality may not have been random with respect to the genotypic variation within the family, in which case the observed plastic responses may be biased in favour of those associated with the genotypes of the survivors. The restricted diet treatment produced smaller imagines, but it did not result in precocious male emergence. Instead, underfed males (on both host-plants) emerged later than full-fed ones (on *A. petiolata*) ($t_7 = 3.56$, $p < 0.01$), at the same time as the females ($t_8 = 0.59$, $p = 0.57$) (Table 6.2). These results indicate that precocious male emergence on *C. pratensis* is not related to their smaller size, and that severe disruption in the developmental process through undernourishment abolishes protandry (i.e. emergence of males before females). Moreover, under-fed specimens suffered reduced longevity compared with full-fed ones (Table 6.3).

Table 6.2. Effect of host-plant, size and nutrition on emergence timing in *A. cardamines*. (Family trait differences are likely genetic; see Table 6.7)

Family	Sex	Host	Treatment	N	Mean LL	Mean WL	Mean Eclosion Day
A	m	Cp	ad lib	4	27.75	19.00	4.50
	m	Ap	ad lib	6	28.67	19.67	6.33
	f	Cp	ad lib	3	30.00	20.00	7.67
	f	Ap	ad lib	3	29.33	20.67	7.33
B	m	Ap	ad lib	4	32.00	20.25	7.75
	m	Ap	restricted	2	26.00	17.00	10.00
	m	Cp	restricted	3	25.00	15.00	11.00
	f	Ap	ad lib	2	32.00	22.50	11.00
	f	Ap	restricted	1	25.00	15.00	11.00
	f	Cp	restricted	2	25.50	14.50	11.00

Cp - *Cardamine pratensis*; Ap - *Alliaria petiolata*. ad lib - larvae supplied with abundant food until pupation; restricted - host-plant withdrawn after larvae reached 25 mm in length. LL - larval-length; WL - wing-length.

Table 6.3 Survival of *A. cardamines* imagines reared on a normal (ad lib) or restricted diet (host-plant withdrawn when larvae reached 25 mm). The normal diet sample has been supplemented with individuals from additional families reared in the same year and environment to those presented in Table 6.2.

Treatment	Mean Emergence Day	N	Mean WL	% Alive on 16 May	% Alive on 19 May
Normal (ad lib)	26 April	31	20.2	97	90
Restricted Diet	28 April	8	15.4	0	0

N = number, WL = wing-length

Longitudinal depression of wing-length on localized spatial and temporal scales

The longitudinal decrease in male wing-length occurred uniformly among sub-sites (Table 6.4, Fig. 6.6), and so is not due to a spatially localized effect. Regression analysis revealed that males were on average smaller at Otters' Tunnel (Table 6.4), which is likely attributable to the occurrence of the smallest *C. pratensis* plants there (Figs. 6.3, 6.5). The parallel decrease in wing-length at this site and in the remaining sub-sites is confirmed when the regression equation is plotted against the annual wing-length averages (Fig. 6.6). Qualitatively similar results were obtained for the females on a more diffuse scale, but the regression model was not significant (Table 6.5).

Table 6.4 Effect of year and Otters' Tunnel (OT) on sub-site average wing-length of male *A. cardamines* in Dibbinsdale Nature Reserve for 2004-2015. Initially all sub-sites and year were entered into the regression model; sub-sites other than OT were removed via backward deletion (outliers >2 standard deviations in a preliminary model were excluded from the analysis).

	Unstandardized Coefficients		Standzd Coeffs	t	p	Correlations			Collinearity Statistics	
	B	SE	Beta			Zero-order	Partial	Part	Tol	VIF
Const	139.2	24.0		5.797	<.001					
Year	-.060	.012	-.461	-4.986	<.001	-.382	-.572	-.458	.986	1.015
OT	-.734	.103	-.656	-7.100	<.001	-.601	-.705	-.651	.986	1.015

dependent variable: Male Wing-length

F = 33.9, p << 0.0001. Adjusted R² = 0.554.

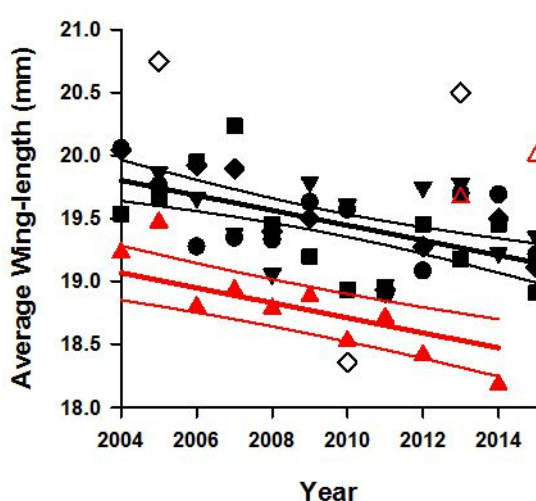


Figure 6.6 Mean male wing-length at individual sub-sites in Dibbinsdale Nature Reserve for 2004-2015, with best-fit lines (and 95% confidence bands) from regression model in Table 6.2 for Otters' Tunnel (red) and all remaining sub-sites combined (black). Symbols represent Spital (black circles), Lower Tip (black triangles), Boden's Hey + Upper Tip + Lady Bridge (combined due to low numbers in some years, black squares), Marford's Wood (black diamonds) and Otters' Tunnel (red triangles). Open symbols show outliers excluded from the regression model.

Table 6.5 Effect of year and North Dibbinsdale (ND = Spital + Otters Tunnel) on sub-site average wing-length of female *A. cardamines* in Dibbinsdale Nature Reserve for 2004-2015.

	Unstandardized Coefficients		Standzd Coeffs	t	p	Correlations			Collinearity Statistics	
	B	SE	Beta			Zero-order	Partial	Part	Tol	VIF
Const	84.3	54.5		1.548	.137					
Year	-.032	.027	-.245	-1.174	.253	-.245	-.248	-.245	1.000	1.000
ND	-.152	.187	-.169	-.813	.425	-.169	-.175	-.169	1.000	1.000

dependent variable: Female Wing-length

F = 1.02, p = 0.378. Adjusted R² = 0.002.

The decrease in male wing-length was more consistent in early than in late season (Fig. 6.7a). For the early season data, the R² value for a simple linear regression

(0.62) is reasonably close to that for a fifth-order polynomial equation ($R^2 = 0.86$), indicating that the decline was relatively uniform. (A polynomial fit will *always* be more accurate than a linear one, but in this case the simple regression holds up reasonably well, indicating that the polynomial equation has captured unwanted noise.) For the late season data, the R^2 value (0.89) for a polynomial equation greatly exceeds that for a simple regression (0.15), indicating that the decline was haphazard. In particular, the late season data exhibits a sudden decline in average wing-length in 2008 and a sharp increase from 2011 to 2013 (Fig. 6.7a). Similar trends are discernible in the data for females, but with less precision (Fig. 6.7b).

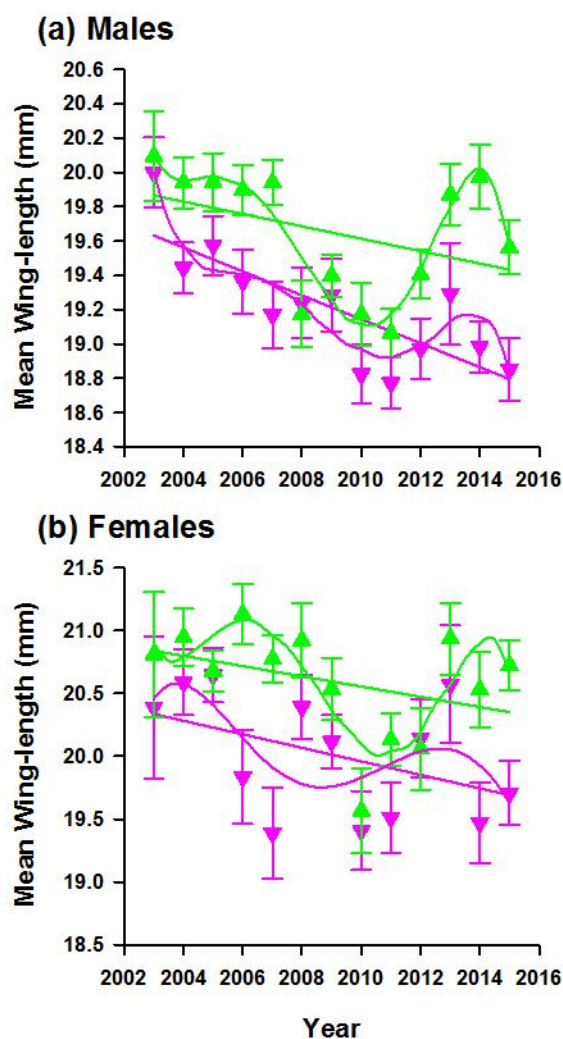


Figure 6.7 Longitudinal changes in early (pink) and late (green) season mean wing-lengths (\pm SE) of male and female *A. cardamines* in Dibbinsdale Nature Reserve. Each series is shown with the best-fit regression line and fifth-order polynomial curve.

The increase in average wing-length after 2011 is likely related to very poor weather conditions in 2012. In that year, males began emerging on 26 March, and from this

date until 11 May inclusive (47 days total), there were only 13 good weather days (28%) on which the butterflies were active. The loss of 34 days in ~1.5 months may have fatally weakened freshly emerging small specimens more often than large ones, since they are unlikely to have the same level of stored energy reserves as the larger insects; small specimens resulting from a restricted diet are certainly weaker than normal sized ones in the laboratory (Table 6.3). The run of poor weather also interfered with egg-laying on small *C. pratensis* (Chapter 5, Fig. 5.2); the resulting host-shift onto large *C. pratensis* and *A. petiolata*, on which larvae grow larger (Chapter 2), may have contributed to the increase in the average wing-length of the adults the following year. On the other hand, the average wing-length declined sharply in 2010 after a high rate of parasitism was recorded on the Wirral in 2009 (Appendix 7), as would be expected if larger larvae are more vulnerable to attack (Chapter 4).

Phenological interaction of male and female *A. cardamines* and *C. pratensis*

The phenologies of male and female *A. cardamines* and *C. pratensis* behaved in a similar way during the period 2004-2015 (Fig. 6.8), except that the variance in the first recorded date for females was about half that for males and *C. pratensis* (Table 6.6). This difference is not significant ($F = 1.98$, $p = 0.14$), but hints that females may be responding to a different set of phenological cues than males or *C. pratensis*, or that their response to the same set of cues is dampened. The phenologies of *A. cardamines* males and *C. pratensis* were highly correlated ($r = 0.86$, $p < 0.001$, neglecting 2008, when snow intervened between the flowering and emergence dates, as an outlier), supporting the idea that they respond to the same set of cues. However, there was no detectable advance in the phenology of *C. pratensis* during the study period (slope of phenology on year = 0.2, $R^2 = 0.006$, $p = 0.81$), as there was for males (Fig. 6.1). On average, males begin to emerge one week earlier than females, and *C. pratensis* begins to flower two weeks before the first emergence of males (Table 6.6).

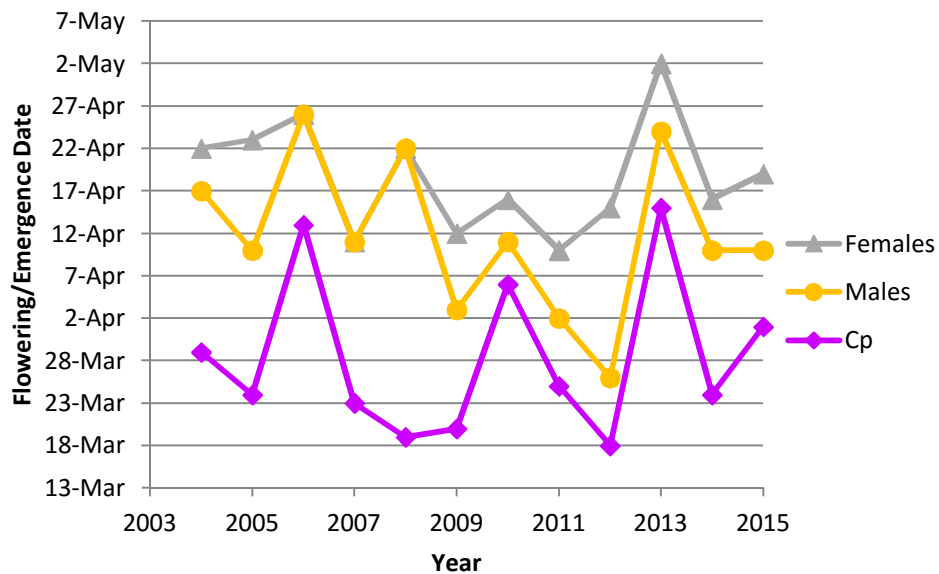


Figure 6.8 Phenology of *C. pratensis* (Cp) as measured by first flowering date, and male and female *A. cardamines*, as measured by first emergence date, in Dibbinsdale Nature Reserve.

Table 6.6 Phenological statistics for first appearance (flowering/emergence) of *C. pratensis*, and male and female *A. cardamines*.

	Mean Date	Earliest Date	Latest Date	Variance
<i>C. pratensis</i>	28 March	18 March	15 April	89.7
Male <i>A. cardamines</i>	11 April	26 March	26 April	86.2
Female <i>A. cardamines</i>	18 April	10 April	2 May	43.5

Two hypotheses can be tested regarding the phenological interaction between male and female *A. cardamines* and *C. pratensis*. The first is that female emergence has responded to selection imposed by *C. pratensis* flowering time. In this case, the gradient of the regression line of emergence day on flowering day is predicted to be 1, since this would enable the butterflies to perfectly track the plants in phenological space. This is not expected *a priori*, since females emerge too late to utilize *C. pratensis* for egg-laying. The regression line (Fig. 6.9a) is highly significant, but its gradient (0.55) differs significantly from 1 ($t = 2.87$, $df = 9$, $p = 0.0185$). This means that females emerge earlier relative to the plants the later they flower (presumably in cold seasons), and hence do not track them consistently on a year to year basis.

The second hypothesis that can be tested is that male emergence and *C. pratensis* flowering have both evolved in response to female emergence timing. This is expected *a priori*, since males should be selected to maximize encounters with virgin females (Chapter 3) and plants should be selected to avoid egg-laying ones (Chapter 5). The regression lines (Fig. 6.9b) are highly significant with gradients (1.05 in both

cases) that do not differ significantly from 1 (for males $t = 0.19$, $df = 9$, $p = 0.85$; for plants $t = 0.17$, $df = 9$, $p = 0.87$). Hence, males and plants can accurately track females in phenological space, as predicted.

It is interesting that *C. pratensis* plants accurately track the first appearance of *A. cardamines* females, but the reverse is not true, since the two regression lines are derived from the same set of data. This is because the variance in plant flowering time is much greater than the variance in female emergence timing (Table 6.6). This extends and contracts the x -axis range when these data sets are respectively used for the independent variable in the regression analysis, causing the gradients obtained to differ widely from one another.

Relative emergence timing of females is clearly related to size (wing-length) in the Dibbinsdale population; when the first emerging females are smaller, they emerge earlier relative to the first flowering date of *C. pratensis* (Fig. 6.10). Hence, the depression of female wing-length over time (Fig. 6.2b) could be a response to selection for egg-laying on *C. pratensis*, since they are currently maladapted to its phenology (Fig. 6.9a). To test this hypothesis, the residuals from the best-fit line of emergence date against flowering date (Fig. 6.9a) were plotted against mean annual wing-length (Fig. 6.11). The results confirm that the negative residuals which represent early emergence relative to the average reaction norm (best-fit line in Fig. 6.9a) are strongly associated with depressed population-level wing-lengths; as the mean size of the females gets smaller, their phenology advances relative to that of *C. pratensis*. The same result holds for the males (Fig. 6.11; residuals obtained from best-fit line shown in Fig. 6.9a). Since there is no explanation as to why this sex should be encroaching on the phenology of a host-plant, it is likely that the females are driving the advance and that the male response is correlated with it. (The better fit for the male data is probably due to better sampling.)

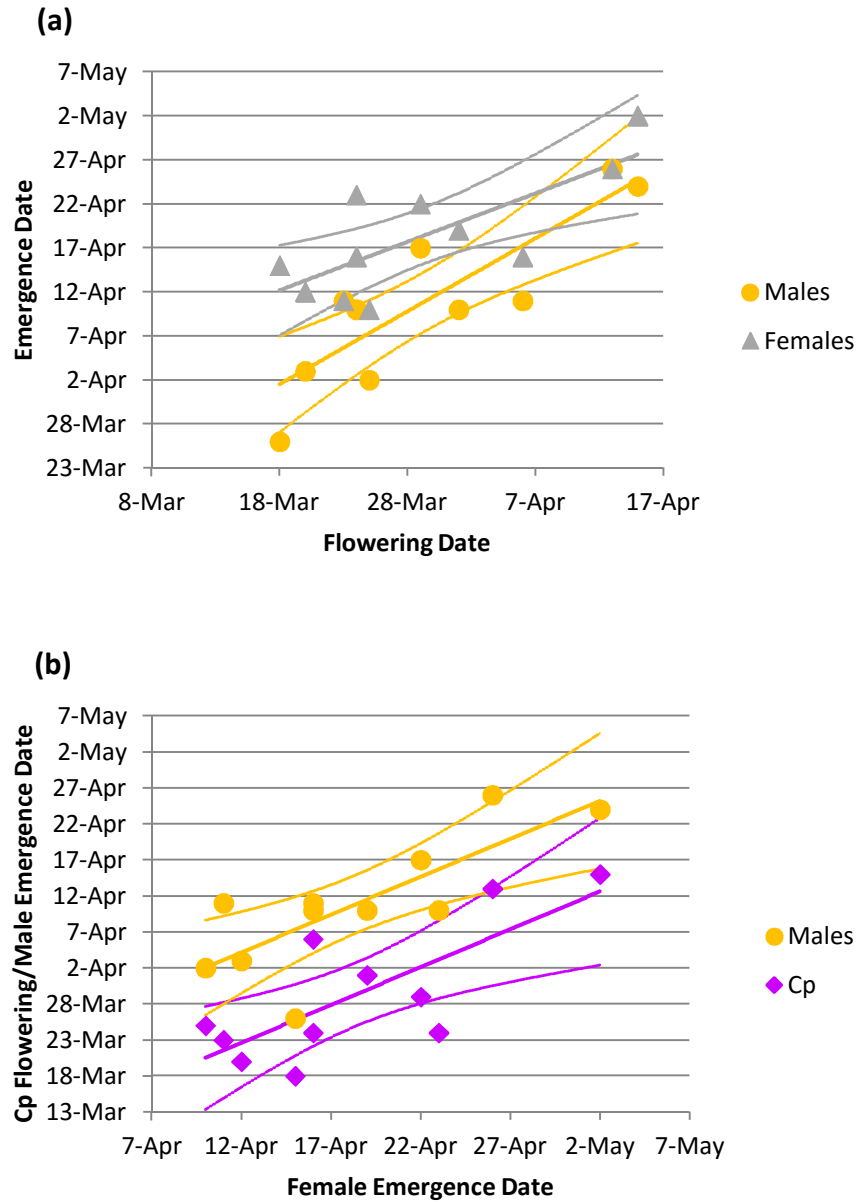


Figure 6.9 (a) *A. cardamines* emergence date as a function of *C. pratensis* flowering date (with 95% confidence bands). Male and female regression equation gradients (\pm S.E.) = 0.83 ± 0.17 , 0.55 ± 0.16 ; $R^2 = 0.73$, 0.58 ; $p = 0.0008$, 0.0066 respectively. (b) *C. pratensis* (Cp) flowering date and male *A. cardamines* emergence date as a function of female *A. cardamines* emergence date. Cp and male regression equation gradients = 1.05 ± 0.30 , 1.05 ± 0.27 ; $R^2 = 0.58$, 0.62 ; $p = 0.0066$, 0.0041 respectively.

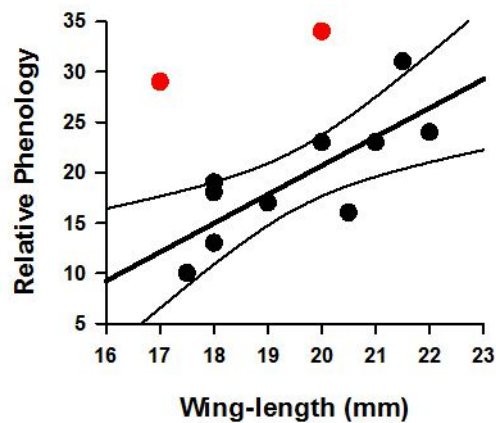


Figure 6.10 Regression (with 95% confidence bands) of relative phenology (number of days after first *C. pratensis* plant recorded in flower) on mean wing-length of females caught on first day of (female) emergence: relative phenology = $2.86 \times \text{wing-length} - 36.6$, $R^2 = 0.61$, $p = 0.0074$. (Red symbols are regarded as outliers and were excluded from the regressions.)

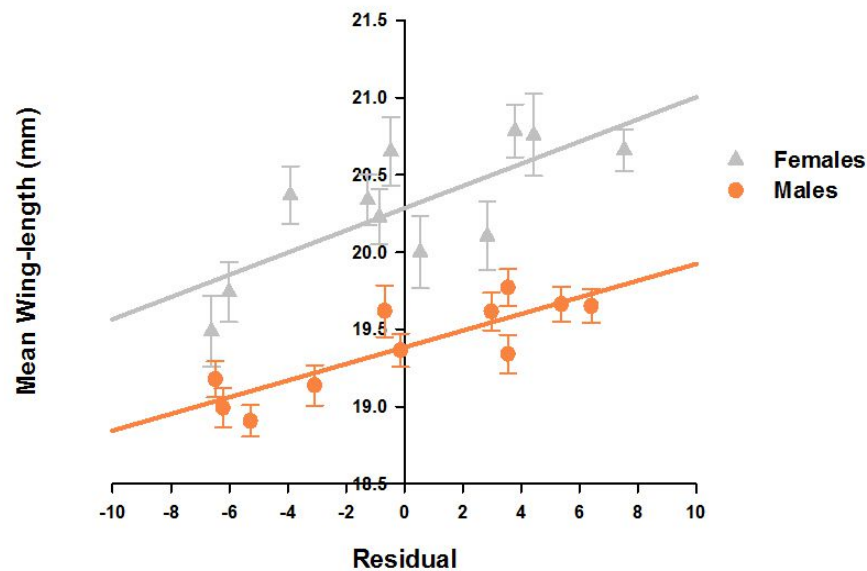


Figure 6.11 Regression of mean annual *A. cardamines* wing-length (\pm SE) on the residuals from the emergence date on flowering date best-fit lines (Fig. 6.9a). Females: $WL = 0.0719 \times R + 20.285$, $R^2 = 0.56$, $p = 0.008$. Males: $WL = 0.054 \times R + 19.385$, $R^2 = 0.73$, $p = 0.0009$. [WL = wing-length, R = residual.]

Heritability of *A. cardamines* wing-length and eclosion date

The heritabilities of wing-length, eclosion day, and the genetic correlation between them, were significant for laboratory reared *A. cardamines* collected on the Wirral peninsula in 2009-2012 (Table 6.7). Estimates of the genetic correlation did not differ between the sexes when males and females were analyzed separately. Hence,

there is a strong positive genetic correlation between size and emergence date in both sexes; larger specimens emerge later. The within-trait intersexual genetic correlations for wing-length and emergence date were positive and significant, but the between-trait correlations were not significant (Table 6.8).

Table 6.7 Heritability of *A. cardamines* wing-length, eclosion day, and the genetic correlation between them (21 families, 197 specimens, from stock collected on the Wirral peninsula in 2009-12).

Trait	h^2	95% C.I.		p
		Lower Bound	Upper Bound	
Wing-length	0.43	0.25	0.61	<0.001
Eclosion Day	0.27	0.06	0.48	<0.05
Genetic Correlation	0.47	0.26	0.68	<0.01

Table 6.8. Inter-sexual genetic correlations for wing-length and eclosion day (SE in parentheses).

		Male Trait	
		Wing-length	Eclosion Day
Female Trait	Wing-length	0.70*** (0.17)	0.32 (0.23)
	Eclosion Day	0.12 (0.24)	0.56* (0.20)

** *p < 0.001, * p < 0.05.

Discussion

Synchronized annual changes in mean *C. pratensis* plant-height at separate sub-sites within Dibbinsdale (Fig. 6.3) indicate that plants in different populations respond in a similar way to widespread abiotic factors, most likely temperature or rainfall. Consistent differences in relative plant-height among sub-sites (Fig. 6.3) could be due to more localized abiotic factors (e.g. soil nutrient content), local adaptation, or founder effects (since clonal proliferation would allow large populations of ramets to be derived from a small number of genets). In the case of the large ecotype growing at the Upper Tip, genetic differentiation is likely, since it remains morphologically distinct when grown in a common garden with the small ecotype (H. McAllister, *pers. comm.*).

Full-grown *A. cardamines* larvae vary positively in size (body-length) with mean sub-site *C. pratensis* plant-height, indicating that nutrition is limiting on this host-plant. In butterflies, it is usual for small specimens to emerge later than large ones (M. Singer, *pers. comm.*; Carvalho, Queiroz and Rusczyk, 1998), suggesting that the optimal developmental response cannot be achieved on a limited diet. However, small *A. cardamines* males which have utilized *C. pratensis* emerge earlier than large

ones which have utilized *A. petiolata* (Davies and Saccheri, 2013; Chapter 2), suggesting that the ancestral response to nutrient limitation has been modified in this species. This is supported by the loss of protandry in males reared on a restricted diet, which shows that a nutritional threshold is required for normal development. If this threshold is not reached, males can pupate, but the mortality rate is high and the developmental path leading to early emergence is disrupted (i.e. they are highly stressed). This was probably the ancestral response to undernourishment on *C. pratensis*.

In view of these results, it is proposed that adaptive phenotypic plasticity associated with *A. cardamines* utilization of *C. pratensis* has evolved in four steps:

1. The initial plastic responses were depressed body-size, physiological stress, late emergence and impaired dispersal. These were chance effects, and do not represent an evolutionary response.
2. Maintenance of oviposition on *C. pratensis* due to the advantages associated with depressed dispersal (males) in dense localized populations (Chapter 2) and small size (both sexes) in environments with high rates of parasitoid attack (Chapter 4). Plastic traits which chance to be advantageous are likely crucial for allowing an organism to persist in a novel environment (in this case, the ecological niche associated with *C. pratensis*) long enough to allow directional selection to take place (Ghalambor et al., 2007).
3. Evolution of stress tolerance by genetic canalization of the developmental response on *C. pratensis* (Grether, 2005; Ghalambor et al., 2007). This would effectively lower the nutritional threshold required for normal development and hence restore protandry.
4. Selection for enhanced protandry due to depressed dispersal of small males, as outlined in Appendix 2.

Exposure to novel environments is likely to trigger a wide range of plastic responses, some of which will be advantageous and others disadvantageous; if possible, selection will reduce the plasticity of disadvantageous traits and increase that of advantageous ones in the direction of the optimum response (West-Ebehard, 2003; Ghalambor et al., 2007). In this case, plasticity associated with physiological stress

has been reduced, that associated with body-size and dispersal rate has been retained, while that associated with emergence timing has been optimized. Since there is free interchange between *A. cardamines* demes utilizing *C. pratensis* and *A. petiolata*, and since high dispersal rates are predicted to oppose local adaptation and promote adaptive phenotypic plasticity (Scheiner, Barfield and Holt, 2012), genetic assimilation of the plastic response has not occurred. Instead, it has been genetically *accommodated* through selection for enhanced plasticity (West-Eberhard, 2003).

The concurrence of the advancement of first emergence date and the depression in mean wing-length (Figs. 6.1 and 6.2) suggests these phenomena are linked. The weight of the evidence points to an evolutionary response in which females are emerging earlier to better utilize *C. pratensis* for oviposition. In the laboratory, the heritabilities of wing-length and emergence date are moderate to high and their genetic correlation is strong (Table 6.7). In nature, the first emerging females are earlier relative to *C. pratensis* when small (Fig. 6.10), and there is a strong relationship between the average female wing-length in the Dibbinsdale population and the residuals from the regression of female emergence on flowering time (Fig. 6.11). The tendency of these residuals to be negative in recent seasons suggests that the reaction norm of emergence date on flowering date, which is currently maladapted to track *C. pratensis*, is changing. The more consistent depression of wing-length in early than in late season (Fig. 6.7) supports the hypothesis that selection is occurring with respect to *C. pratensis* (and not *A. petiolata*). Hence, *A. cardamines* females appear to be responding to the selection pressure imposed by early flowering *C. pratensis*.

A. cardamines males and *C. pratensis* plants have almost certainly been selected to accurately track the emergence of *A. cardamines* females (Fig. 6.9). This is not surprising, since their interaction with females profoundly affects their fitness: males must emerge optimally to mate with them, and *C. pratensis* plants must avoid egg-laying. If, however, females are responding to early flowering *C. pratensis*, then both these relationships could be affected. With respect to *C. pratensis*, this emphasizes the oscillatory form of temporal dynamics associated with antagonistic coevolutionary interactions (Thompson, 2005). At the moment, the (small ecotype of) *C. pratensis* has the upper hand over *A. cardamines*, insofar as it is very successful at avoiding egg-laying (Chapter 5). However, if *A. cardamines* females

continue to invade its phenological space, then the current balance of well-adapted plants and maladapted butterflies will change. If so, then the coevolutionary interaction between them will have escalated.

Selection for early emerging females will automatically select for early emerging males, since the inter-sexual genetic correlation for this trait is strong (Table 6.8). In the field, males emerge earlier relative to the first flowering date of *C. pratensis* when the average population wing-length is depressed, as do females (Fig. 6.11). Since the inter-sexual genetic correlation between emergence date and wing-length is weak (Table 6.8), selection for early emerging females would not automatically select for smaller males. However, early emerging males should themselves be selected for depressed dispersal and hence small size. Hence, as a working hypothesis, it is proposed that direct selection on female emergence timing is accompanied by correlated responses in female wing-length and male emergence timing, leading to direct selection on male wing length. The whole process is being driven by selection for oviposition on *C. pratensis*.

The most obvious fitness gains resulting from oviposition on *C. pratensis* will accrue to males, since the phenotypically plastic "emerge early and wait" mate location strategy associated with its utilization will be beneficial in the high density Dibbinsdale population (Davies and Saccheri, 2013; Chapter 2). On the other hand, females utilizing *C. pratensis* are likely to suffer reduced fecundity (Chapter 4). Hence, oviposition preference may be subject to sexually antagonistic selection; if so, this could limit the extent to which the butterfly invades the plants' phenological space. However, if depressed larval size on *C. pratensis* protects both sexes from parasitoid attack (Chapter 4), the effects of sexual antagonism will be reduced or overturned.

The first flowering date of *C. pratensis* has not advanced during the study period (Fig. 6.8). Since male *A. cardamines* emergence timing and *C. pratensis* flowering date are strongly correlated (Fig. 6.8) and have similar variances (Table 6.6), they likely respond to the same phenological cues. If so, the detectable advance in the first appearance date of male *A. cardamines* (Fig. 6.1) can be solely attributed to the correlated response with selection on female emergence timing. This cautions against interpreting the long-term advancement in *A. cardamines* phenology solely in terms

of an unmodified response to climate change. However, good weather conditions in early season will likely be critical if the butterfly is to move into *C. pratensis*' phenological space; inclement weather in 2012 was probably responsible for a temporary reversal in the longitudinal decline in the size of the imagines in the Dibbinsdale population (Fig. 6.7). Global warming may therefore have an important indirect role to play in determining the outcome of the current phase in the oscillating coevolutionary interaction between *A. cardamines* and *C. pratensis*.

Taken together, the results presented in this Chapter suggest that pre-existing phenotypic plasticity in developmental responses can act as a starting point for adaptive evolutionary change; the initial reaction norm of *A. cardamines* larvae to *C. pratensis* utilization has likely changed, and that of the imagines to seasonal temperatures may be in the process of changing. This supports current ideas on the evolution of adaptive phenotypic plasticity (e.g. West-Eberhard, 2003; Ghalambor et al., 2007). It is interesting to compare these ideas with Fisher's theory of dominance (Fisher, 1930). In both cases, an initial response, to a new environment or to a new gene, produces a wide range of effects, or a "mosaic of traits" (Ghalambor et al., 2007), some of which are advantageous and others disadvantageous. Selection then acts on the gene-complex to enhance the advantageous effects and depress the disadvantageous ones, producing adaptive phenotypic plasticity and dominance, respectively. This emphasizes the similarity between the developmental processes underlying phenotypic plasticity and polymorphism, to which attention has recently been drawn (Wennersten and Forsman, 2012; Forsman, 2015).

Chapter 7

Discussion

Geographical mosaics are driven by varying selection pressures affecting coevolutionary interactions across landscapes (Thompson, 2005). Some of the selection pressures operating on the tritrophic interaction between *Anthocharis cardamines*, its host-plants *Cardamine pratensis* and *Alliaria petiolata*, and the parasitic fly *Phryxe vulgaris* in the Dibbinsdale population, and the likely response of these organisms to them, are as follows.

1. *C. pratensis*

Selection pressure: High-level pre-dispersal seed predation by *A. cardamines* larvae.

Evolutionary response:

Small ecotype: Phenological escape (Fig. 7.1), with most plants flowering early (before the emergence of the butterfly), especially large ones which are particularly vulnerable to oviposition; a small fraction flower later when the butterfly is scarce. The resultant bimodal flowering curve is strongly suggestive of the outcome of disruptive selection.

Large ecotype: Decreased effective conspicuousness, with individual plants selected to avoid flowering in peak season, when oviposition rates are high due to increased visual conspicuousness of host-plant patches. The result is a broad, unpeaked flowering curve (Fig. 7.1).

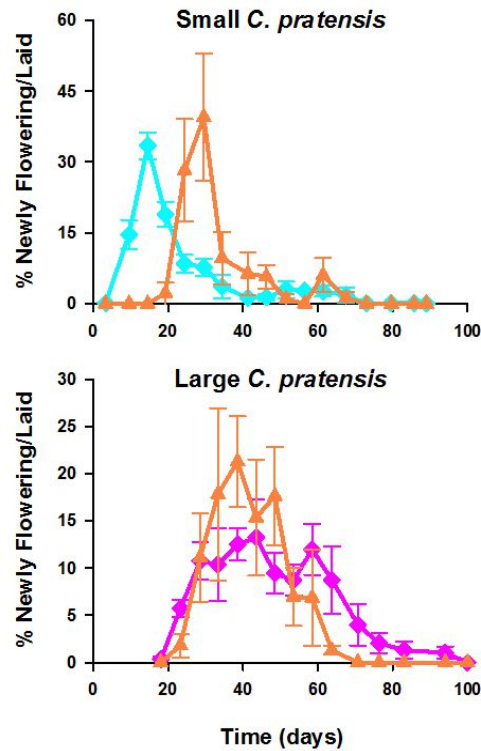


Figure 7.1 Flowering curves of small (cyan) and large (pink) *C. pratensis* plants, with *A. cardamines* egg-laying curves superimposed (orange). Data points are the means (\pm SE) for transects in the Dibbinsdale population over the four year period 2011-14. The small ecotype mostly flowers in advance of the emergence of the butterfly, thereby escaping its egg-laying activity; the broad, unpeaked flowering curve of the large ecotype reduces egg-laying activity by decreasing the visual conspicuousness of plant patches from a distance.

2. *A. petiolata*

Selection pressure: Low-level pre-dispersal seed predation by *A. cardamines* larvae.

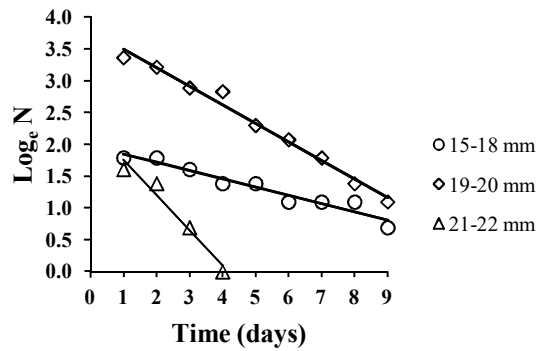
Evolutionary response: None. The plants' fitness is too little affected by the butterfly to exert a significant selection pressure.

3. *A. cardamines*

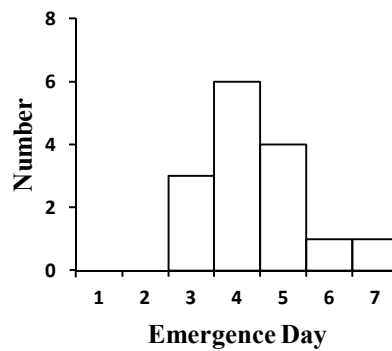
Selection pressure 1: Depressed size on small ecotype of *C. pratensis*.

Evolutionary response 1: Adaptive phenotypic plasticity. The developmental response has been canalized to reduce physiological stress associated with sub-optimal nutrition. Reduced size is stabilized by depressed male dispersal. Protandry has increased in response to male philopatry (Fig. 7.2) in localized high-density populations, facilitated by the patchy distribution of *C. pratensis* across the landscape. However, depressed female fecundity likely remains a disadvantage.

(a) Dispersal



(b) Emergence on Cp



(c) Emergence on Ap

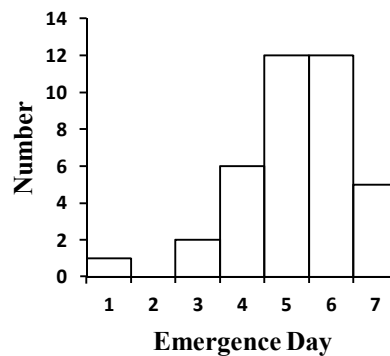


Figure 7.2 Interaction between male size, dispersal rate, host-plant and emergence timing in the Dibbinsdale population. (a) Small males disperse more slowly than larger ones; in this residence plot from 2007, the log number of specimens remaining in the population declines more slowly with time for smaller males. Laboratory reared males are smaller (not shown) and (b) emerge earlier (mean \pm 95%CI = 4.4 ± 0.6 d) when fed on *C. pratensis* than (c) when fed on *A. petiolata* (5.4 ± 0.4 d). Since slow dispersal favours protandry, the smaller size of specimens utilizing *C. pratensis* has likely selected for earlier male emergence on that host.

Selection pressure 2: Reduced mating opportunities for males utilizing *A. petiolata* in high density populations due to the earlier emergence of competitors utilizing *C. pratensis*. (A consequence of the plasticity described above.)

Evolutionary response 2: Stay-or-go dispersal, in which late emerging males either vacate the habitat immediately, or remain within it (Fig. 7.3). The former response is made feasible by the reduced importance of early male emergence for mate location in low-density populations. The latter response is likely frequency-dependent, since the loss of competitors via dispersal will boost the fitness of individuals that stay behind. While it is unlikely that the response is host-plant specific, the later emergence of males utilizing *A. petiolata* will increase the likelihood of a "go" response, when their larger size and hence faster dispersal will be an advantage in searching for widely scattered females.

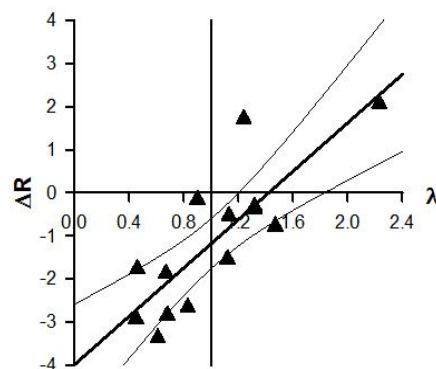


Figure 7.3 Departure from predicted number of male recaptures (ΔR) against predicted male fitness (λ) in the Dibbinsdale population. The data points correspond to different intra-seasonal periods within the years 2005-2010. Positive/negative values of ΔR indicate that more/less males were recaptured than predicted; predicted fitness indicates the average number of matings a male can expect. Negative values of ΔR , indicating a recapture deficit, are associated with low predicted fitness in late intra-seasonal periods (regression equation: $\Delta R = 2.81\lambda - 3.99$, $R^2 = 0.67$, $p = 0.0004$; regression line shown with 95% confidence bands) and are interpreted as due to rapid dispersal from the population (the "go" response) when the chances of obtaining a mating are low.

*Selection pressure 3: Early flowering of small *C. pratensis*.*

Evolutionary response 3: Small, early emerging females are currently being selected to oviposit on this host (Fig. 7.4); however, they are still poorly adapted to its phenology.

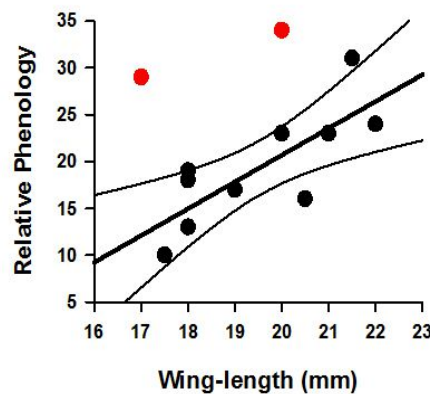


Figure 7.4 Smaller *A. cardamines* females emerge earlier relative to *C. pratensis* than large ones; hence, current selection of small females will enable them to invade *C. pratensis* phenological space. Regression (with 95% confidence bands) of relative phenology (number of days after first *C. pratensis* plant recorded in flower) on mean wing-length of females caught on first day of emergence: relative phenology = $2.86 \times \text{wing-length} - 36.6$, $R^2 = 0.61$, $p = 0.0074$. (Red symbols are regarded as outliers and were excluded from the regression.)

Selection pressure 4: High rates of larval parasitism by *P. vulgaris*.

Evolutionary response 4: Selection of small specimens which are (probably) less conspicuous and (certainly) spend less time exposed on the host-plant. Evolution of a behavioural polymorphism in which some larvae (possibly in response to environmental cues) vacate the host-plant before the final instar resting phase. Maintenance of oviposition on the large ecotype of *C. pratensis* which supports the fastest larval growth-rate; or the evolution of a fast growth-rate on this host due to a high attack rate.

4. *P. vulgaris*

Selection pressure: Specialization on *A. cardamines*.

Evolutionary response: Not directly researched, but the fly's synchronization with *A. cardamines*' life cycle is suggestive. *P. vulgaris* is usually bivoltine, but when utilizing *A. cardamines* it is frequently univoltine (Courtney and Duggan, 1983). All nine specimens reared from *A. cardamines* larvae collected on the Wirral (Appendix 7) emerged the following spring, and therefore omitted the summer brood.

The inter-relations between the adaptations and counter-adaptations of the organisms in this tritrophic system are summarized in Fig. 7.5.

Phryxe vulgaris

synchronized life cycle

Anthocharis cardamines

emerge late & rove

♂ dispersal ↑

♀ fecundity ↑

large body-size

large size, thin continuous distribution

Alliaria petiolata

small size at pupation

vacate host before resting phase

rapid development

pre-dispersal seed predation

stay or go

emerge early and wait

small early emerging ♀♀

♀ fecundity ↓

♂ dispersal ↓

small body-size

small size, dense localized distribution

small *Cardamine pratensis*

early flowering

broad flowering curve

large *Cardamine pratensis*

Figure 7.5 Traits and adaptations in the tritrophic interaction between the autotrophs *Alliaria petiolata* (colour-coded green), small (mauve) and large (purple) *Cardamine pratensis*, the herbivore *Anthocharis cardamines* (orange), and the parasitoid *Phryxe vulgaris* (grey) in the Dibbinsdale area. Dashed arrows show the consequences of trait plasticity; solid arrows the outcome of evolutionary change (but note that some of the plastic changes may themselves have been modified by selection). *A. petiolata* is a large host-plant producing large *A. cardamines* larvae; as a consequence, the resultant male imagines are fast dispersers and the female imagines have high fecundity. Rapid male dispersal and the thin continuous distribution of *A. petiolata* across the landscape have selected for an emerge late and rove mate location strategy. Food limitation on small *C. pratensis* results in small *A. cardamines* larvae; the resultant male imagines are slow dispersers and the female imagines have low fecundity. A combination of depressed male dispersal and the dense localized distribution of small *C. pratensis* across the landscape has selected for an emerge early and wait mate location strategy. The two alternative mate location strategies have selected for the stay-or-go response, in which disadvantaged late emerging males (likely to be associated with *A. petiolata*) redistribute themselves across the landscape. High levels of pre-dispersal seed-predation by *A. cardamines* larvae has selected for early flowering small *C. pratensis*, which avoid egg-laying *A. cardamines* females (phenological escape); however, females are currently being counter-selected to emerge early to oviposit on this host, at least partly due to the advantages accruing to their sons, although they will suffer depressed fecundity due to their small size. Pre-dispersal seed predation has also selected for the broad flowering curve exhibited by large *C. pratensis*, in which individual plants avoid egg-laying females as far as possible by flowering off peak-season. High rates of larval parasitism inflicted on *A. cardamines* larvae by *P. vulgaris* maintains selection for small size at pupation (in which case male dispersal and female fecundity will be depressed), and has selected for a behavioural polymorphism in which some larvae leave the host-plant before the final instar resting phase (reducing exposure to the fly), and rapid larval development (associated with large *C. pratensis*). Some strains of *P. vulgaris* are synchronized with *A. cardamines*' life cycle, indicating close adaptation to its phenology.

All the adaptations and counter-adaptations described above could change among populations across landscapes, and within populations through time, facilitating the formation and evolution of geographic selection mosaics. Spatial dynamism is implied by the shift in *A. cardamines* host use across the British mainland (Courtney and Duggan, 1983), and by its more polyphagous strategy in Sweden (Wiklund and Åhrberg, 1978). The small and large ecotypes of *C. pratensis* in Dibbinsdale employ different phenological strategies to avoid egg-laying *A. cardamines* females, while *C. pratensis* plants in some localities in Sweden are phenologically vulnerable and have evolved increased tolerance to larval damage (Boalt *et al.*, 2010) or are more reliant on high larval mortality (Wiklund and Friberg, 2009). Similarly, the tachinid fly *P. vulgaris* takes a heavy toll on British *A. cardamines* populations (Courtney and Duggan, 1983), whereas in some parts of Sweden its place is taken by the ichneumonid *Cotesia saltator* (Wiklund and Åhrberg, 1978; Wiklund and Friberg, 2009). These parasitoids attack different larval stages, with *C. saltator* infecting the early instars and *P. vulgaris* the later ones; counter-adaptations to the one would not

therefore match counter-adaptations to the other. Temporal dynamism is implied by the invasion of (small) *C. pratensis*' phenological space by *A. cardamines* females; if continued, this will alter the current balance of advantages and disadvantages between the host-plant and herbivore.

The terms 'coevolutionary hotspot' and 'coevolutionary coldspot' distinguish cases in which selection is reciprocal from those in which it acts on only one of the interacting species (Thompson, 2005). In the Dibbinsdale population, the reciprocal interaction between *A. cardamines* and (small) *C. pratensis* qualifies as a coevolutionary hotspot; this may be true also of the interaction between *A. cardamines* and *P. vulgaris*. Interestingly, the selection of small, early emerging female *A. cardamines* to oviposit on small *C. pratensis* should act agonistically with the selection of small butterflies (both sexes) to avoid parasitism. In this case, the coevolutionary dynamics within the hotspot may be relatively simple, since the middle organism in the tritrophic interaction (*A. cardamines*) will not have to trade-off its responses to lower and higher trophic levels. On the other hand, the interaction between *C. pratensis* and *A. cardamines* in Sweden is more likely to be a coevolutionary coldspot, since the evolution of tolerance in the host-plant is unlikely to be coupled with selection on the butterfly, since in that country it is highly polyphagous (Wiklund and Åhrberg, 1978), and may be regarded as a phenological specialist (laying eggs on whichever host-plant is in the correct phenological stage) rather than a host-plant specialist (Posledovich et al., 2014; Navarro-Cano et al., 2015). It is possible that *C. pratensis* provides a partial refuge from *C. saltator* (Wiklund and Friberg, 2009); if so, this may help maintain oviposition on this host, although the interaction is likely weak.

Spatio-temporal dynamism leading to the formation of coevolutionary hotspots and coldspots exemplify the existing theory of geographic selection mosaics (Thompson, 2005). Three novel aspects of selection mosaics to emerge from the study of the interactions between *A. cardamines*, its host-plants and *P. vulgaris*, are: the occurrence of coevolutionary responses in a wider ecological context; carryover effects of coevolutionary adaptations on intra-specific interactions; and phenotypic plasticity.

The earlier emergence of male *A. cardamines* utilizing *C. pratensis* is ultimately dependent on the uneven distribution of the plants across the landscape (Davies and Saccheri, 2013). Since *C. pratensis* is usually abundant in, but restricted to, damp habitats, it tends to be associated with high-density, localized *A. cardamines* populations, in which protandry is favoured. On the other hand, *A. petiolata* is more widely distributed owing to its occurrence along hedgebanks and lanes, and therefore tends to be associated with low-density, continuous *A. cardamines* populations, in which protandry is less favoured. Hence, host-plant species acts as a cue for the type of population in which males are likely to emerge. This is not a typical coevolutionary response, in which one organism is selected in relation to the phenotypic traits of another. However, it has required genetic canalization of the developmental response on *C. pratensis*, which is more typical of coevolutionary interactions. The coevolutionary response is therefore embedded in a wider ecological context in which male *A. cardamines* are adapted to the changing availability of mates across heterogeneous landscapes.

Host-driven plasticity in emergence timing affects intra-specific competition in habitats with mixed hosts. Late emerging males which have utilized *A. petiolata* will be on average at a disadvantage compared with earlier emerging ones which have utilized *C. pratensis* (Davies and Saccheri, 2015). In the Dibbinsdale area, this has likely assisted the evolution of the 'stay-or-go' response, in which late emerging males in the source population are either philopatric or dispersive. Hence, carryover effects from coevolutionary interactions between species have the potential to modify selection pressures acting on intra-specific interactions within them. The outcome could affect, or be affected by, the structure of the associated geographic mosaic. In *A. cardamines*, the 'go' response redistributes males across the landscape, which should counter Allee effects in low density areas and reduce genetic differentiation between demes. However, it will only be effective in areas where high density populations are immersed in a low density continuum; in the absence of a continuum, selection on emergence timing should be stronger, as appears to be the case in Durham.

Phenotypic plasticity has the potential to adapt organisms to local conditions on a much finer scale than genetic differentiation. Geographic selection mosaics exist at a variety of scales, with trans-continental mosaics being the largest (Thompson, 2005).

The smallest scale will usually be determined by how rapidly the coevolutionary interaction between organisms changes across the landscape relative to their dispersal kernels. However, phenotypic plasticity does not depend on genetic differentiation, and so dispersal will not interfere with it. I shall introduce the term 'micro-mosaic' to describe geographical variation in coevolutionary interactions on a scale determined by environmental cues rather than genetic differentiation. The plastic response of *A. cardamines* to its host-plants in Dibbinsdale exemplifies such a micro-mosaic, since phenotypic variation which would otherwise require a high degree of genetic isolation to evolve occurs within a very small area (475 ha); small specimens resulting from plasticity on *C. pratensis* at the Otters' Tunnel sub-site are about the same size as the typical form adapted to the same host-plant in Ireland (Majerus, 1979).

The phenological shift in *A. cardamines* emergence timing in response to early flowering *C. pratensis* emphasizes the need for greater understanding of the evolutionary ecology of organisms responding to climate change. In general, butterflies are responding to global warming faster than plants (Parmesan, 2007), suggesting that phenological mismatching between these trophic levels may be a common outcome. If, however, butterflies are responding more quickly due to selection for earlier emergence, then either phenological matching trades-off with other important factors, or the assumption that these herbivores are currently well matched to their host-plants is incorrect. Ecosystem functioning will partly be determined by the nature of the coevolutionary interactions between trophic levels within foodwebs (Thompson, 2009). Hence, the occurrence of geographic selection mosaics implies that ecosystem functioning is partly dependent on rapid evolutionary adjustments and counter-adjustments even in the absence of climate change. The onset of global warming therefore offers the opportunity to study the role of adaptation in the resilience of ecosystems to disturbances in trophic structure. The tritrophic interaction between *C. pratensis*, *A. cardamines* and *P. vulgaris* comprises a good model system for such work. Future efforts should focus on continued monitoring of the phenological response of *A. cardamines* to *C. pratensis*' flowering time in Dibbinsdale, further elucidation of the genetics of *A. cardamines*' life-history traits and of the *C. pratensis* ecotypes, a detailed investigation of whether the synchronization of *P. vulgaris*' and *A. cardamines*' life cycles is indicative of cryptic

speciation in the former, and analyzing changes in the trophic interactions across the landscape.

Appendices

Appendix 1

To show that the resettlement-rates imply that small specimens were less dispersive than larger ones in the WS in 2006 and 2007.

Start with the residence-rate, which is really the product of the true survival-rate, φ , and the complement of the emigration-rate, Ψ , (i.e. $1 - \Psi$), which will be termed the retention-rate, ρ (rate at which living specimens are retained in an area—a measure of their sedentariness). So

$$\text{residence rate} = \varphi \cdot \rho$$

Now if in the SSFC (denoted F), the residence-rates of small (S) and large (L) butterflies are the same (condition 1), then

$$\begin{aligned} \varphi_S \cdot \rho_S^F &= \varphi_L \cdot \rho_L^F \\ \frac{\varphi_S}{\varphi_L} &= \frac{\rho_L^F}{\rho_S^F} \end{aligned} \tag{A1.1}$$

where the sub-scripts denote the size of the butterflies, and the superscript denotes the spatial scale (note that survival is independent of scale, so there's no superscript for φ). The resettlement-rate (r) is just the residence-rate in the WS (denoted W) minus the residence-rate in the SSFC; therefore, the ratio of the resettlement-rates for the small and large specimens is

$$\frac{\varphi_S (\rho_S^W - \rho_S^F)}{\varphi_L (\rho_L^W - \rho_L^F)} = \frac{r_S}{r_L}$$

Substituting from (A1.1) and rearranging

$$\frac{\rho_S^W - \rho_S^F}{\rho_S^F} = \frac{r_S}{r_L} \left(\frac{\rho_L^W - \rho_L^F}{\rho_L^F} \right)$$

Now if $r_S > r_L$ (condition 2) then

$$\frac{\rho_S^W}{\rho_S^F} - 1 > \frac{\rho_L^W}{\rho_L^F} - 1$$

$$\rho_S^W > \frac{\rho_S^F}{\rho_L^F} \rho_L^W$$

Therefore the WS retention-rate for the small specimens will be greater than that for the large ones provided that $\rho_S^F \geq \rho_L^F$, that is, the SSFC retention-rate of the small specimens is greater than or equal to that of the larger ones (condition 3). If this is the case, then

$$\rho_S^W > \rho_L^W$$

$$\psi_L^W > \psi_S^W$$

The large specimens are dispersing from the WS faster than the small ones.

Appendix 2

Evolution of emergence timing in isolated populations in response to depressed dispersal-rate

The coupling between depressed dispersal and advanced eclosion in small male *A. cardamines*, which have utilized small *C. pratensis*, is interesting in view of the mathematical relationships derived to explain protandry, the prior eclosion of males before females. Thus, the number of matings expected for a male emerging at time t , $\varphi(t)$, which can be taken to be directly proportional to their fitness, is

$$\varphi(t) = \int_t^T s^{(z-t)} \frac{f(z)}{M(z)} dz$$

where $f(t)$ is the female emergence-curve (number of females emerging at time t), and $M(t)$ is the number of males (competitors) alive at time t , given by

$$M(t) = \int_0^t s^{(t-z)} m(z) dz$$

where $m(t)$ describes the male emergence-curve; s is the daily survival-rate, and 0 and T represent the start and end dates of the flight period, respectively. (Modified from Bulmer (1983) and Iwasa *et al.* (1983); a similar equation was derived by Parker and Courtney (1983)).

Here I consider the conditions under which an ESS for male emergence time is invadable by a male mutant with an alternative strategy. Since the mutant is rare, its effect on the number of competitors, $M(t)$, will be negligible whenever it emerges. However, from the equation for $\varphi(t)$, its relative fitness will be given by the ratio of

its survival-rate, s , to that of the common form. So, a mutant male with a higher survival-rate would be able to invade the population.

Now, in isolated populations, emigration will act analogously to death, since dispersing males will be unable to find a mate. For simplicity, I will consider a situation in which host use affects dispersal-rate (as it does in *A. cardamines*), but that all other causes of mortality are unaffected; in this case, a difference in survival-rate between the two hosts will reduce to a difference in dispersal-rate. If a population is established on a host producing fast-dispersers (survival-rate s_1), what will happen if a mutant form arises which utilizes an alternative host producing slow-dispersers (survival-rate s_2)?

After the slow-dispersers gain a footing in the population, they will begin to affect the number of competitors, $M(t)$. Let us assume that their emergence-curve is given by $m_2(t)$, and that $m_1(t)$ represents the original emergence-curve of the fast-dispersers. (For simplicity, I will assume that the female emergence-curve, $f(t)$, remains unchanged.) Now if the slow-dispersers have a tendency to emerge late in the season (starting at k , where $k > 0$), then the number of females mated by a slow-disperser emerging at time t (where $t \geq k$) is

$$\varphi_2(t) = \int_t^T s_2^{(z-t)} \frac{f(z)}{\int_0^z s_1^{(z-y)} m_1(y) dy + \int_k^z s_2^{(z-y)} m_2(y) dy} dz$$

Since $s_2 > s_1$ and the denominator is the same for the two competing dispersal morphs (i.e. the equation for $\varphi_1(t)$ is the same as that for $\varphi_2(t)$ except that s_2 (in the numerator) is replaced by s_1), then $\varphi_2(t) > \varphi_1(t)$. This means that as the generations pass the second term in the denominator will increase at the expense of the first term as the slow-dispersers replace the fast-dispersers in late season ($t \geq k$). This in turn will lead to a progressive decline in $\varphi_2(t)$ as the slow-dispersers increasingly come into competition with other slow-dispersing morphs.

However, the number of females mated by a mutant slow-disperser emerging earlier than k , say at j (i.e. $j < k$) is

$$\varphi_2(j) = \int_j^k s_2^{(z-j)} \frac{f(z)}{\int_0^z s_1^{(z-y)} m_1(y) dy} dz + \int_k^T s_2^{(z-j)} \frac{f(z)}{\int_0^z s_1^{(z-y)} m_1(y) dy + \int_k^z s_2^{(z-y)} m_2(y) dy} dz$$

That is, the mutant has access to an earlier period in the season which is free of its slow-dispersing co-competitors; since it outcompetes the fast-dispersing competitors, $\varphi_2(j) > \varphi_1(j)$, it is able to ‘invade’ this earlier period. Moreover, during the early part of this invasion, $\varphi_2(j \leq t \leq k) > \varphi_2(k \leq t \leq T)$, since slow-dispersing co-competitors are relatively rare in the period between j and k . Therefore, the slow dispersers will be selected for early emergence, with a corresponding shift in $m_2(t)$.

Provided that the requisite genetic variation is available to advance the emergence time still further, the model predicts that the process will continue until the slow-dispersers have replaced the fast-dispersers in the population (effected through a change in host utilization). Once this has happened, an ESS of emergence times among the slow-dispersers could evolve; since their residence-time is prolonged, they would emerge earlier on average than the fast-dispersers did (Fig. 2.8, main text).

Appendix 3

Critique of Courtney's (1982b) hypothesis on the maintenance of polyphagy in *A. cardamines*.

Courtney (1982b) suggested that the apparently maladaptive preference of female *A. cardamines* for host-plants on which larval-survivorship is low (*Hesperis matronalis* and *Barbarea vulgaris*) in an isolated population in Durham, northern England, could be explained as an adaptive response to time-limitation. On this hypothesis, the total search-time available to females to find hosts is short and prevents them from depositing their full egg-load; females encountering *any* host on which larval survival is possible should therefore oviposit. Since the poorest hosts happen to be the most ‘apparent’ (conspicuous), they receive most eggs in spite of their unsuitability (Courtney, 1982a).

Courtney's hypothesis is relevant here since the implication is that time-limitation is sufficient to explain polyphagy in *A. cardamines* (Courtney suggested that his model

would be applicable to all *A. cardamines* populations). However, Courtney's own data shows that oviposition in Durham was *not* indiscriminate: out of 106 encounters with *A. petiolata* plants, females were observed to oviposit on only 13 occasions (Courtney, 1982a, Table 3). This is not consistent with time-constrained behaviour, and indicates that the low realized fecundity (egg-shortfall) in this population was at least partly due to a high oviposition rejection-rate. Therefore, the question as to why females did not reject unsuitable hosts in this locality resurfaces.

As it stands, the assumption that *H. matronalis* and *B. vulgaris* were unsuitable due to poor larval survivorship neglects the fact that these hosts depressed pupal (and hence by implication imaginal) size (Courtney, 1981). Therefore, if the results reported here are generally applicable, males utilizing these hosts will exhibit depressed dispersal. Courtney (1981) states that there was little or no interchange of specimens between his study populations, indicating that they were isolated; moreover, the males in the Durham locality were "extremely localized" (Courtney and Duggan, 1983) in their behaviour. Hence depressed dispersal should be selected for in this locality. I suggest that this potential small-male advantage could trade-off against poor larval-survival and reduce, or even overturn, the apparent unsuitability of *B. vulgaris* and *H. matronalis* as hosts. Thus, in Dibbinsdale in 2007, the smallest males had a residence time 4.3 times longer than the largest ones; if similar effects occurred in Durham, the impact on male reproductive success may have been considerable. In summary, the extension of the consequences of host use to the imaginal stage indicates that female oviposition behaviour in Durham may be less maladaptive than it first appears.

In Britain generally, *C. pratensis* and *A. petiolata* are by far the most commonly utilized hosts. In Dibbinsdale, larval-survivorship is high and very similar on these two plants, and this was also the case in Courtney's populations. Therefore, the problem of maladaptive oviposition behaviour, whereby preference is inversely related to larval-survival, does not arise in relation to these hosts. (I am here assuming that small *C. pratensis* is the most common form of this plant nationally, as it is in Dibbinsdale.) I suggest that the maintenance of these two plants in *A. cardamines*' host range is at least partly due to balancing selection associated with the contrasting adult life-history traits resulting from their utilization, and find the

observed coupling of depressed dispersal and early eclosion on *C. pratensis*, as well as national trends in host use, encouraging for this hypothesis.

Appendix 4

Derivation of predicted fraction of recaptures, F

For any population of regularly sampled mobile living organisms, the number of recaptures depends on two parameters: the residence-rate (describing the proportion of specimens retained in the population between sampling occasions) and the encounter-rate (describing the proportion of specimens resident in the population which are encountered on each sampling occasion). The residence-rate determines the average period (called the residence time) an individual remains in the population; it is directly proportional to the survival-rate and inversely proportional to the emigration-rate. The encounter-rate determines the average time (recapture period) between successive encounters (captures) of the same individual during its residency in the population. Intuitively, the longer the residence time, and the shorter the recapture period, the more individuals will be recaptured at least once.

The residence-rate can be estimated from MRR data by plotting the number of specimens remaining in the population against time. The encounter data for the whole season are synchronized by setting the day of first capture for all individuals to zero; a specimen is regarded as resident in the population every day until the day of last capture. If the decline is exponential (as is usual for insect populations), then a logarithmic transformation will yield a straight line graph (here termed the residence plot, Fig. A4.1). The gradient (m) of this line gives the residence-rate as $\exp(m)$ and the residence time as $-1/m$ (where the minus sign corrects for the negative value of m). The encounter-rate can be found in a similar way by plotting the number of recaptures remaining against time, in which all recaptures are synchronized by setting the date of previous release to zero. For example, a specimen recaptured 1, 4, 8 and 10 days after initial capture would contribute four recaptures to the total number, which after synchronization would occur after 1, 3, 4, and 2 days. Provided the decay is exponential, a logarithmic transformation will produce a straight line graph (the recapture plot, Fig. A4.1), whose gradient (g) yields the encounter-rate as $[1 - \exp(g)]$ (since $\exp(g)$ gives the rate at which specimens *avoid* recapture) and the recapture period as $-1/g$.

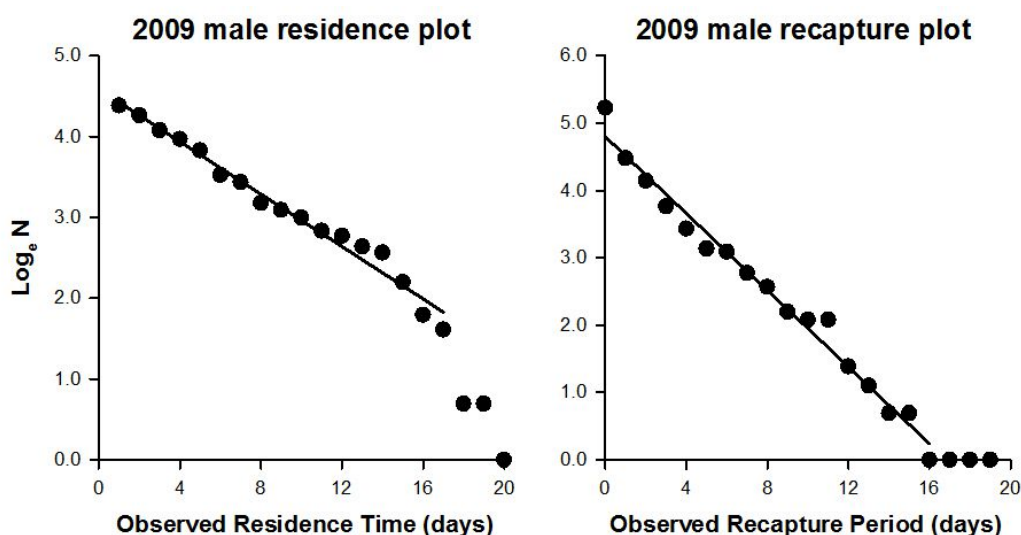


Figure A4.1 Male residence plot and recapture plot for the 2009 season. N = number of specimens/recapture events remaining with (synchronized) time for the residence/recapture plots, respectively. For gradients of the best-fit lines, see under m and g in Table 3.1, main text.

Since insect populations are usually sampled on a daily basis, the gradients m and g express the logarithmic decline in the number of specimens/recaptures *per day*; hence, the *daily* residence-rate and the *daily* encounter-rate are calculated from them. It is possible to express these parameters in alternative time units by modifying their formulae to $\exp(mt)$ and $[1 - \exp(gt)]$ respectively, where t indicates the desired time unit in days. For example, the residence-rate of specimens over half-day and two-day intervals is given by $\exp(m/2)$ and $\exp(2m)$ respectively.

Having obtained m and g from the residence and recapture plots, the fraction of specimens predicted to be recaptured at least once can be calculated. I first *exclude* recaptures obtained on the day of initial capture, since it is very easy to recapture a specimen within minutes of its release, and therefore very difficult to decide on a convention as to which recaptures to include as 'genuine'. Hence, I confine the analysis to recaptures made from the day after release (day 1) onwards.

Let

N = total number of specimens captured (taken to occur on day 0).

n = actual number of specimens in the population on day 1.

n' = known number of specimens in the population on day 1, equal to the total number of specimens eventually recaptured.

F = fraction of specimens recaptured ($= n'/N$).

s = daily residence-rate ($= e^m$).

p = daily encounter-rate ($= 1 - e^g$).

If N specimens are caught on day 0 then the number remaining on day 1, n , will be

$$n = Ne^{m/2}$$

where I have assumed that specimens are (on average) first encountered half way through day 0, so that the proportion remaining on day 1 is found by substituting $t = 1/2$ into $\exp(mt)$. Of these

$$np = e^{m/2} Np$$

are actually captured.

Therefore $e^{m/2} Np$ provides the first contribution to the total number of specimens recaptured (n'). Having made this contribution, these specimens are now redundant and in order to avoid counting them twice they must be subtracted from n , to give the number of unrecorded specimens remaining after day 1 as

$$e^{m/2} N(1 - p)$$

To obtain the number of unrecorded specimens remaining on day 2, this is multiplied by s ; to obtain the number caught on day 2, the result in turn is multiplied by p ; therefore the second contribution to n' is

$$e^{m/2} N(1 - p)sp = (e^{m/2} Np)[(1 - p)s]$$

Repeating this process, the third contribution to n' is

([Number of specimens present on day 2] – [Number of specimens caught on day 2])
x s x p

$$= (e^{m/2} N(1 - p)s - e^{m/2} N(1 - p)sp)sp$$

$$= (e^{m/2} N(1 - p)s)(1 - p)sp = (e^{m/2} Np)(1 - p)^2 s^2$$

$$= (e^{m/2} Np)[(1 - p)s]^2$$

Inspection of the first three contributions to the total number of specimens recaptured, viz $e^{m/2} Np$, $(e^{m/2} Np)[(1 - p)s]$, and $(e^{m/2} Np)[(1 - p)s]^2$ indicates that they

form the first three terms of a Geometric Progression, with first term $e^{m/2}Np$ and common ratio $(1-p)s$. Therefore summing the terms to infinity

$$n' = \frac{e^{m/2}Np}{1-(1-p)s}$$

and the total fraction of specimens recaptured is

$$F = \frac{e^{m/2}p}{1-(1-p)s}$$

This equation gives the predicted value of F in terms of the daily residence-rate (s) and the daily encounter-rate (p). Substituting $s = e^m$ and $p = 1 - e^g$

$$F = \frac{e^{m/2}(1-e^g)}{1-e^g e^m} = \frac{e^{m/2}(1-e^g)}{1-e^{g+m}}$$

As it stands, this equation is not accurate since it was derived from a Geometrical Progression representing the estimated number of recaptures obtained at discrete daily intervals; since sampling effort is continuous, this provides only a crude representation of the recapture process. Therefore, the correct formula for F will only be found by introducing t into the equations for s and p and letting it tend to zero (which effectively converts recapture into a continuous process). Hence

$$F = \frac{e^{m/2}(1-e^{gt})}{1-e^{(g+m)t}}$$

which is insoluble as $t \rightarrow 0$, being of the form $0/0$. It is therefore necessary to employ l'Hôpital's rule and differentiate both numerator and denominator, giving

$$\frac{-e^{m/2}ge^{gt}}{-(g+m)e^{(g+m)t}}$$

which, on letting t tend to zero, gives

$$F = e^{m/2} \frac{g}{g+m}$$

as the correct formula for the fraction of specimens predicted to be recaptured at least once.

An intuitive grasp of this equation can be gained by considering the effects of extreme values of m and g . Thus, as $m \rightarrow 0$, death and emigration will cease and so all specimens will be recaptured eventually ($F \rightarrow 1$); as $m \rightarrow -\infty$, death and emigration will be instantaneous and so no specimens will be recaptured ($F \rightarrow 0$). On the other hand, if $g \rightarrow 0$, encounters will cease and so no specimens will be recaptured ($F \rightarrow 0$); whereas if $g \rightarrow -\infty$, specimens will be encountered immediately and so all available on day 1 will be recaptured ($F \rightarrow e^{m/2}$).

As a bonus an estimate of the size of the population (N_{pop}) being sampled can be obtained from $1/F$ (where the term $e^{m/2}$ is dropped since we do not have to wait an initial period before the specimens become available for capture). Hence

$$N_{pop} = \frac{g + m}{g}$$

It should be noted, however, that this equation is dependent on the assumption of uniform behaviour; if there is a 'stay-or-go' response, such that some specimens emigrate from the population immediately, the estimate obtained will not be accurate.

Appendix 5

Independence of 'stay-or-go' response from body-size (wing-length)

The analysis of plastic dispersal behaviour in the main text (Chapter 3) neglects the possible impact of size on dispersal-rate. In Dibbinsdale, large male *A. cardamines* emerge later and disperse more quickly than small ones (Chapter 2), so it may be questioned whether this effect contributes to the observed decrease in the number of recaptures in late season. I here analyse the results from the 2007 season, since in that year small, medium and large sized males exhibited distinct dispersal-rates (Table A5.1).

Table A5.1 Residence plot gradients (m), and the residence-rates (s), residence times (RT) and predicted fraction of recaptures (F) derived from them for small, medium and large sized male *A. cardamines* in Dibbinsdale in 2007. The values given here are slightly different from those given in Chapter 2 (Table 2.5), since in that Chapter specimens not recaptured in their SSFC within the Reserve were excluded from the analysis to reduce noise in analyzing resettlement rates. The gradient of the recapture plot (g) used to calculate F is given in Table 3.1.

Wing-length (mm)	m	S.E.	s	RT	F
15-18	-0.173	0.014	0.84	5.78	0.756
19-20	-0.287	0.012	0.75	3.48	0.640
21-22	-0.476	0.057	0.62	2.10	0.498

It is found that the 'stay-or-go' response affects all three size categories (Table A5.2); it is not caused by the later emergence of large fast-dispersing specimens as might have been suspected. Hence all wing-lengths suffer a decrease in recapture-rate in late season, such that the actual number of recaptures falls significantly below expectation (indicated by negative ΔR) at that time. This does not mean that size does not affect dispersal-rate in late season, but rather that the 'go' response is independent of it.

Table A5.2 Intra-seasonal changes in recapture-rate for small, medium and large sized male *A. cardamines* in Dibbinsdale in 2007, together with the results of χ^2 tests on the significance of the departures from expectation. N = number of males captured during the specified period, O_R = observed number of recaptures, E_R = expected number of recaptures (= NF , where F is taken from Table A5.1), and ΔR = standardized residual (from equation 3.5, main text).

Period (d)	Wing-length (mm)	N	O_R	E_R	ΔR	χ^2	P
1-15 (early season)	15-18	12	7	9.1	-0.688	1.940	0.181*
	19-20	54	35	34.6	+0.075	0.016	0.901
	21-22	16	8	8.0	+0.011	0.0003	0.987
16-32 (late season)	15-18	7	1	5.3	-1.866	14.266	0.001*
	19-20	22	8	14.1	-1.620	7.293	0.007
	21-22	19	5	9.5	-1.451	4.192	0.041

*Simulated P-values (2000 replicates)

Appendix 6

Variance in source emergence timing and maintenance of sink populations

In order to see how source-sink structure might impact on the evolution of emergence timing through dispersal, I make three key assumptions (my analysis owes a debt to the derivation of Pulliam (1988), but my assumptions are very different).

1. Since we are interested in males only, I equate reproductive success with the number of matings obtained, instead of the more direct measure of the number of offspring produced (this implicitly assumes that all matings are of equal value to males). Since females are monandrous, the number of mating opportunities for males is equal to the number of females. I take the sex-ratio to be equal.
2. In heterogeneous landscapes, I assume two basic types of habitat, determined by population density: high density *sources*, in which emerging males encounter (and mate with) all females, and low density *sinks*, in which emerging males do not encounter all females. (For simplicity, I hereafter consider a single source and sink.)
3. Within the source population there will be dominants and subordinates; that is, some males (dominants) achieve >1 matings and others (subordinates) <1 . I assume that subordinates improve their fitness by emigrating to the sink, although their fitness remains <1 (they are effectively "making the best of a bad job"). In this model, dominance relationships in the source are determined by emergence timing, and subordinates increase their fitness in the sink due to the lower population density (less intense mate competition) there. I assume a simple response in which males emerging in the source either 'stay' (dominants) or 'go' to the sink immediately (subordinates).

Let

n_s = number of 'stay' males emerging in source.

n_g = number of 'go' males emerging in source.

n_m = total number of males emerging in source ($= n_s + n_g$).

n_f = number of females emerging in source ($= n_m$ for an equal sex-ratio).

n = number of males plus females emerging in the source; for an equal sex-ratio $n/2 = n_f = n_m$.

λ_s = average number of matings obtained by 'stay' males in source; $\lambda_s > 1$ by assumption 3.

λ_g = average number of matings obtained by males in sink (whether emerging in source ('go' males) or sink); $\lambda_g < 1$ by assumption 3.

n_{skm}/n_{skf} = total population of males/females *emerging* in sink (for males, before immigration of "go" specimens).

n_{sk} = number of males plus females emerging in the sink; for an equal sex-ratio $n_{sk}/2$
 $= n_{skm} = n_{skf}$.

N = number of males plus females in the whole population (source + sink).

α = proportion of females mated in the sink. (In the source, all females are mated by assumption 2.)

I shall first derive equations giving the number of specimens of each type emerging in the landscape, together with ones describing their reproductive success. Since 'stay' males mate with all the source females:

$$n_f = n_s \lambda_s$$

so

$$\lambda_s = \frac{n_f}{n_s} = \frac{n}{2n_s} \quad (\text{A6.1})$$

or

$$n_s = \frac{n}{2\lambda_s} \quad (\text{A6.2})$$

on the assumption of an equal sex-ratio ($n_f = n_m = n/2$). So

$$n_g = n_m - n_s = \frac{n}{2} - n_s$$

$$n_g = \frac{n}{2} \left(1 - \frac{1}{\lambda_s} \right) \quad (\text{A6.3})$$

Since sink females are mated either by males emerging in the sink or by 'go' males emerging in the source:

$$n_{skm} \lambda_g + n_g \lambda_g = \alpha n_{skf}$$

$$\lambda_g = \frac{\alpha \frac{n_{sk}}{2}}{\frac{n_{sk}}{2} + n_g} \quad (\text{A6.4})$$

$$\frac{n_{sk}}{2} (\lambda_g - \alpha) = -n_g \lambda_g$$

$$n_{sk} = \frac{2n_g \lambda_g}{(\alpha - \lambda_g)} \quad (\text{A6.5a})$$

$$n_{sk} = \frac{n\left(1 - \frac{1}{\lambda_s}\right)\lambda g}{\alpha - \lambda g} \quad (\text{A6.5b})$$

Note from equation (A6.4) that $\lambda g < \alpha$, so the denominator in (A6.5b) is positive. The total number of specimens is:

$$\begin{aligned} N &= 2(n_s + n_g) + n_{sk} \\ &= \frac{n}{\lambda_s} + \left(n - \frac{n}{\lambda_s}\right) + \frac{n\left(1 - \frac{1}{\lambda_s}\right)\lambda g}{\alpha - \lambda g} \\ &= \frac{n(\alpha - \lambda g) + n\left(\lambda g - \frac{\lambda g}{\lambda_s}\right)}{\alpha - \lambda g} \\ N &= \frac{n\left(\alpha - \frac{\lambda g}{\lambda_s}\right)}{\alpha - \lambda g} \end{aligned} \quad (\text{A6.6})$$

These equations give the numbers and fitnesses of specimens emerging in different parts of the landscape, but they neglect the cause of the 'stay-or-go' response, i.e. the assumption that source 'go' males improve their fitness in the sink (assumption 3). If we assume that the *predicted* fitness in the source (*before* the emigration of 'go' specimens) is normally distributed (for justification see below), then we can express the proportion of males whose predicted source fitness falls below the average sink fitness in terms of the cumulative probability function of the normal distribution. This is expressed in terms of the standard variable Z , such that the probability that Z lies below some standardized value of interest, z , is

$$\Phi(z) = \Pr(Z < z)$$

$$z = \frac{x - \mu}{\sigma}$$

where μ is the mean, σ the standard deviation and x the actual value of interest. In our case, we are interested in the fraction of males in the source (where the mean predicted fitness is 1 by assumption 2: since males emerging in the source mate with

all the source females, a 1:1 sex-ratio (assumption 1) guarantees that on average each male gains 1 mating) with predicted fitness below λg ; so

$$z = \frac{\lambda g - 1}{\sigma}$$

where σ is the standard deviation in the predicted fitness of males emerging in the source. Hence, $\Phi((\lambda g - 1)/\sigma)$ will give the probability that source males will have a predicted fitness below λg ; assuming that these emigrate to the sink, we can rewrite the number of 'stay' and 'go' males as

$$n_s = n_m \left(1 - \Phi \left(\frac{\lambda g - 1}{\sigma} \right) \right) = \frac{n}{2} \left(1 - \Phi \left(\frac{\lambda g - 1}{\sigma} \right) \right) \quad (\text{A6.7})$$

$$n_g = n_m \Phi \left(\frac{\lambda g - 1}{\sigma} \right) = \frac{n}{2} \Phi \left(\frac{\lambda g - 1}{\sigma} \right) \quad (\text{A6.8})$$

Substituting (A6.8) in (A6.5a), the total number of males and females emerging in the sink is

$$n_{sk} = \frac{n \left(\Phi \left(\frac{\lambda g - 1}{\sigma} \right) \right) \lambda g}{\alpha - \lambda g} \quad (\text{A6.9})$$

which can be rearranged to give

$$\Phi \left(\frac{\lambda g - 1}{\sigma} \right) = \frac{n_{sk} (\alpha - \lambda g)}{n \lambda g} \quad (\text{A6.10})$$

If we now assume that the variance in predicted source male fitness ($= \sigma^2$) is caused by departures from an ESS distribution of emergence times, this equation expresses the relationship between precision in male emergence timing and the size of a sink population in heterogeneous landscapes. Since $\lambda g < 1$, $(\lambda g - 1)/\sigma$ is negative and therefore Φ increases with increasing σ ; hence, the sink population is boosted by increasing σ or decreasing precision in emergence timing. Conversely, a large sink population could maintain high σ and hence relieve selection pressure for precise emergence timing; or, if such precision is beyond the evolutionary reach of the organism, the existence of a sink could allow maladaptively emerging males to boost their fitness in a way that would otherwise be unavailable to them.

It is important to be clear that the variance in predicted fitness described by σ^2 refers *only* to that caused by departures from an ESS emergence schedule (i.e. by imperfect emergence timing); if the emergence curve is an ESS (perfect emergence timing), then *all* males are predicted to obtain 1 mating and hence $\sigma^2 = 0$. That is, the additional variance due to sampling error is not included in σ^2 . (In the field, mating success should be Poisson distributed, so departures from an ESS should result in intra-seasonal changes in the mean/variance of this distribution; incorporation of this effect is beyond the scope of the current analysis.) The assumption that imperfect emergence timing leads to a normal distribution in predicted fitness values over the course of the whole season can be tested for the Dibbinsdale population by plotting the frequency of the values obtained from equation 3.3 (main text) over the six-year study period. The result is a reasonable approximation to a Gaussian distribution with $\mu = 1$ and $\sigma = 0.46$ (Fig. A6.2), so this assumption may be accepted.

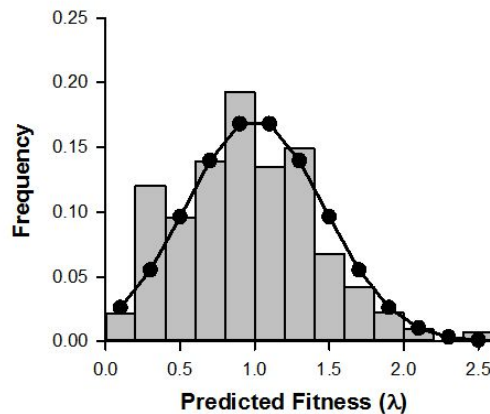


Figure A6.2 Histogram showing the distribution in predicted male fitness values over the six-year study period (2005-2010), which has a standard deviation of 0.46. The Gaussian distribution with $\mu = 1$ and $\sigma = 0.46$ (filled circles) is provided for comparison.

I have assumed that some females in the sink remain unmated ($\alpha < 1$). This is in line with known Allee effects in many organisms (decreasing population growth with decreasing population size due to low mate encounter rates). If, however, an equilibrium is reached in which emigrating males from the source, coupled with those emerging in the sink, mate with all sink females, then we may set $\alpha = 1$ in the above equations (as has been done in the main text). In this case, a 'stay-or-go' response resulting from imprecise emergence timing in the source would be responsible for maintaining a stable population in the sink, which would otherwise

require repeated repopulation through emigrating source females to avoid extinction through Allee effects.

Appendix 7

Threshold larval size for parasitoid attack and variable infection rates in Cheshire

Wild *A. cardamines* larvae were collected from localities in and around Dibbinsdale on the Wirral peninsula in 2009 and 2011. Their date of collection and pupation were noted, allowing a relationship to be derived between time to pupation and rate of infection with *P. vulgaris*. Those collected in 2009 showed a progressively higher rate of parasitization by *P. vulgaris* with age, as judged by time to pupation (Table A7.1); this effect was highly significant ($\chi^2 = 7.78$, simulated p-value (2000 replicates) = 0.017). Larval instar was not recorded, but it is almost certain those collected 0-5 days from pupation were in the 5th instar; those collected 6-10 days from pupation may have been in the 4th or 5th instar. These results suggest that the infection rate of pupating larvae was ~50% in 2009 (specimens were collected from a wide area; the high rate of infection is not due to clumped sampling), with the majority of larvae being parasitized in the fifth instar; the threshold size for vulnerability to attack was probably reached late in the fourth instar. Larvae collected in 2011 were much less heavily parasitized and older larvae were not progressively more vulnerable to attack (Table A7.1); the two stung specimens were collected in the 4th and 5th instar. Hence, the rate of parasitization and the vulnerability of older specimens fluctuates markedly between years; when attack rates are high, older, larger larvae can be very vulnerable.

Table A7.1 Rate of parasitization by *P. vulgaris* of wild *A. cardamines* larvae collected on the Wirral peninsula in 2009 and 2011.

Days to Pupation	2009			2011		
	N	P	%	N	P	%
0-5	5	5	50	8	1	11
6-10	12	2	14	5	1	17
11-21	9	0	0	1	0	0

N = Not Parasitized, P = Parasitized, % = Percentage Parasitized.

Appendix 8

Derivation of equations (4.9) and (4.12) in Chapter 4

(i) Derivation of equation (4.9) for $\ln(S_T)$ when parasitism is restricted to the fifth instar

Start with equation (4.7) in Chapter 4:

$$\ln(S_T) = T \ln(k) + x \int_0^{\alpha T} \ln(L_e) dt + x \int_{\alpha T}^T \ln(L_{5r}) dt = T \ln(k) + x \left[\int_0^{\alpha T} \ln(L_e) dt + \int_{\alpha T}^T \ln(L_{5r}) dt \right]$$

Concentrating for the moment on the terms in parentheses and substituting equations (4.4) and (4.5) for L_e and L_{5r} :

$$\begin{aligned} \int_0^{\alpha T} \ln(D \exp[Ct]) dt + \int_{\alpha T}^T \ln\left(\frac{T-d}{c}\right) dt &= \int_0^{\alpha T} [\ln(D) + Ct] dt + \int_{\alpha T}^T \ln\left(\frac{T-d}{c}\right) dt \\ &= \left[\ln(D).t + \frac{Ct^2}{2} \right]_0^{\alpha T} + \left[\ln\left(\frac{T-d}{c}\right).t \right]_{\alpha T}^T = \ln(D).\alpha T + \frac{C\alpha^2}{2} T^2 + \ln\left(\frac{T-d}{c}\right).(1-\alpha)T \end{aligned}$$

Hence

$$\ln(S_T) = T.\ln(k) + x \left(\frac{C\alpha^2}{2} T^2 + \left[\alpha \ln(D) + (1-\alpha) \ln\left(\frac{T-d}{c}\right) \right] T \right)$$

Now substituting equation (4.8) for $\ln(k)$:

$$\ln(S_T) = x \left(\frac{C\alpha^2}{2} T^2 + \left[(\alpha - 1) \ln(D) + (1-\alpha) \ln\left(\frac{T-d}{c}\right) \right] T \right)$$

Rearranging gives equation (4.9):

$$\ln(S_T) = x \left(\frac{C\alpha^2}{2} T^2 + (1-\alpha) T \ln\left(\frac{T-d}{cD}\right) \right)$$

(ii) Derivation of equation (4.12) for $\ln(S_T)$ when parasitism extends over the fourth and fifth instars

Start with equation (4.11) in Chapter 4:

$$\ln(S_T) = (M + T) \ln(k) + x \left[\int_0^{\beta M + \alpha T} \ln(L_e) dt + \int_{\beta M}^M \ln(L_{4r}) dt + \int_{M + \alpha T}^{M+T} \ln(L_{5r}) dt \right]$$

Concentrating on the terms in parentheses and substituting equations (4.4) and (4.5) for L_e and L_{5r} :

$$\begin{aligned}
& \int_0^{\beta M + \alpha T} [\ln(D) + Ct] dt + \int_{\beta M}^M \ln(L_{4r}) dt + \int_{M + \alpha T}^{M+T} \ln\left(\frac{T-d}{c}\right) dt \\
&= \left[\ln(D) + \frac{Ct^2}{2} \right]_0^{\beta M + \alpha T} + [\ln(L_{4r}) \cdot t]_{\beta M}^M + \left[\ln\left(\frac{T-d}{c}\right) \cdot t \right]_{M + \alpha T}^{M+T} \\
&= (\beta M + \alpha T) \ln(D) + \frac{C(\beta M + \alpha T)^2}{2} + (1 - \beta)M \ln(L_{4r}) + (1 - \alpha)T \ln\left(\frac{T-d}{c}\right)
\end{aligned}$$

Inserting this expression back into equation (4.11), substituting equation (4.8) for $\ln(k)$ and rearranging gives equation (4.12):

$$\ln(S_T) = x \left(\frac{C(\beta M + \alpha T)^2}{2} + (1 - \beta)M \ln\left(\frac{L_{4r}}{D}\right) + (1 - \alpha)T \ln\left(\frac{T-d}{cD}\right) \right)$$

Appendix 9

To determine whether the disappearance of final instar larvae before the resting phase is due to early vacation of the host-plant or predation

First, note that *all* larvae observed to enter the resting phase *must* have survived any threat of predation during the growth phase. The "naive" distribution of the duration of the growth phase for these 141 larvae is shown in Fig. A9.1 (grey bars). If we add the growth phase durations of the 27 larvae which were not observed to enter the resting phase to this distribution, we obtain the "modified" distribution for all 168 larvae shown in Fig. A9.1 (off-white bars).

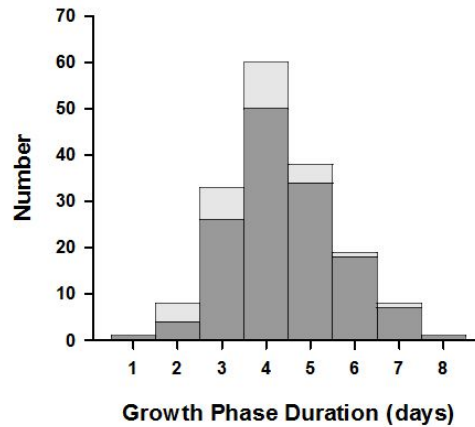


Figure A9.1 Final instar growth phase duration of *A. cardamines* larvae in Dibbinsdale, excluding specimens not observed to enter the resting phase ("naive" distribution, grey), or including these specimens ("modified" distribution, off-white).

Now assume as a null hypothesis that the modified distribution has been caused by predation acting on the naive distribution. We want to know what level of predation will best transform the naive into the modified distribution. As with tachinid attack, the instantaneous daily survival-rate is given by

$$S_t = kL^x = k(De^{Ct})^x$$

where the symbols are as defined in the main text. In this case, however, we simplify the analysis by replacing the specific growth curves for different sized larvae (C variable) with an average growth curve (C constant) such that the average size of a larva in the population depends only on the time it has spent in the growth phase (t), an approximation which is clearly correct (Fig. 4.3) This allows us to make the following rearrangement

$$St = kD^x(e^{Cx})^t = kA^t$$

where k and A are constants and D^x has been absorbed into k ; the rate of predation will be size-independent (and hence time-independent) if $A = 1$, and size/time-dependent if $A < 1$. Since all specimens recorded as entering the 5th instar are ~ 1 day old, and we are only interested in predation occurring after this, we make the following modification:

$$St = 1 \text{ at } t = 1$$

$$St = kA^{t-1} \text{ for } t > 1$$

Starting with the naive distribution (scaled up to 168 specimens), we can apply these equations to see how the loss of specimens before the termination of growth would modify it. For example, of the total number of specimens which spend 4 d in the growth phase in the *absence* of predation (N_4), only $N_4.(1.kA^1.kA^2.kA^3) = N_4.k^3A^6$ would be observed to do so in the *presence* of predation. Of the losses, $N_4.(k^2A^3 - k^3A^6)$ would have been observed to spend 3 d in the growth phase (i.e. the difference between the numbers recorded as present on days 3 and 4); these losses must therefore be added to the total number of specimens which spend 3d in the growth phase in the presence of predation (N'_3). The full formula for obtaining the modified numbers observed on day n (N'_n) from the unmodified ones (N_n) is

$$N'_n = N_n k^{n-1} A^{n(n-1)/2} + \sum_{m=n+1}^T N_m \left(k^{n-1} A^{n(n-1)/2} - k^n A^{(n+1)n/2} \right)$$

where T is the duration of the longest growth period (for my data $T = 8$). The total number of predations (P) is given by

$$P = \sum_n \sum_{m=n+1}^T N_m \left(k^{n-1} A^{n(n-1)/2} - k^n A^{(n+1)n/2} \right)$$

The procedure is to vary k and A to see which values give the best approach (as measured by the χ^2 value) to the modified distribution (Fig. A9.1, off-white bars); on the null hypothesis that the difference between the naive and modified distributions is due to predation, the predicted number of predations (P) should equal the number of larvae which were not observed to enter the resting phase.

The parameter values which best transform the naive into the modified distribution are $k = 1$, $A = 0.994$, for which $P = 8.1$, far short of the 27 larvae which were not observed to enter the resting phase (the difference between the observed and expected numbers in the "predated" and "non-predated" classes is very highly significant: $\chi^2 = 46.46$, $df = 1$, $p = 9.35 \times 10^{-12}$). This is for a size-dependent predation model; if we impose the restriction that predation is size-independent ($A = 1$) then the best value of k is 0.992 for which $P = 4.5$ ($\chi^2 = 114.45$, simulated p-value (2000 replicates) = 0.0005), again far short of the observed value. Similar results are obtained if the naive and modified distributions for the years 2008-11 and 2013 (which differ significantly from each other, Fig. 4.4) are analyzed separately: in every case the predicted number of predations falls significantly short of the observed number of larvae which did not enter the resting phase.

The implication is that the 27 larvae which did not enter the resting phase have *not* been drawn from the same population responsible for the naive distribution by the process of predation, since there are too many of them. They must therefore belong to a separate naive distribution, and hence to a different phenotype: one which passes through the growth phase more quickly on average as well as vacating the host-plant before the resting phase.

Appendix 10

Evolution of *C. pratensis* flowering time in response to selection by egg-laying *A. cardamines* females: a model

To simulate the evolution of flowering time in response to egg laying, a model population of 80 plants was divided into three size categories: 20 small, 40 medium and 20 large. Each plant entered the population (began flowering) in one of seven time periods, with 1, 2, 4, 6, 4, 2, 1 small and large plants entering the population in each period, and double the number of medium sized plants (Fig. A10.1a). The small plants had three flowers per plant when first flowering, and zero flowers thereafter; the medium plants had six flowers per plant when first flowering, two in the succeeding period, and zero thereafter; the large plants had nine flowers per plant when first flowering, four in the first succeeding period, one in the second succeeding period, and zero thereafter. The total number of flowers in each time period was calculated by summing the number of flowers on each plant in flower during that period.

The simulation was undertaken by allowing plants in a specific flowering period within each size category to compete with plants in other flowering periods but not with plants in different size categories. This maintained the number of plants in each size category at their initial values (20 small, 40 medium and 20 large). The fitness of plants in a specific size category (F_s) which flowered in time period t was calculated from:

$$F_s(t) = 1 - \frac{T_f(t)}{480S}$$

where the second term represents a fitness penalty in which $T_f(t)$ is the total number of flowers present on all plants in the population (all size categories) in period t , 480 is the maximum number of flowers which could possibly be present in one period (= $20 \times 3 + 40 \times 6 + 20 \times 9$ if all plants flower in the same period; this factor prevents the fitness ever decreasing below zero) and S is a factor which modulates the fitness penalty for different sized plants ($S = 1$ for large, 2 for medium and 4 for small plants, so that the penalty is halved and then halved again as plants get successively smaller). The model therefore captures the depression of fitness in *C. pratensis* due to the egg-laying activity of *A. cardamines* females, where the number of eggs laid is proportional to both the number of flowers present on the whole transect and to the

size of the individual plants (since oviposition is largely restricted to newly flowering plants, the fitness penalty is acquired at the time of flowering; the model neglects the effects of eggs laid later).

Since fitness is always depressed by egg-laying, the populations in each size category would crash to zero if they were not rescued in each generation by ensuring that the total number of plants across all flowering periods remains constant. This was done by first calculating the average fitness of the plants in a specific size category ($F_{av,s}$) over the whole season:

$$F_{av,s} = \frac{\sum_t N_s(t) \cdot F_s(t)}{\sum_t N_s(t)}$$

where $N_s(t)$ is the number of plants entering the population at time t ; then obtaining the relative fitness ($F_{rel,s}$) of plants in each time period:

$$F_{rel,s}(t) = \frac{F_s(t)}{F_{av,s}};$$

and finally obtaining the number of plants in each time period in the succeeding generation $N_{(g+1),s}$ from the number in the current generation $N_{(g),s}$:

$$N_{(g+1),s}(t) = N_{(g),s}(t) \cdot F_{rel,s}(t)$$

where in all cases the subscript s denotes a specific size category. Hence, the number of plants in a specific size category flowering in each time period changes in response to selection, but the total number over all time periods does not.

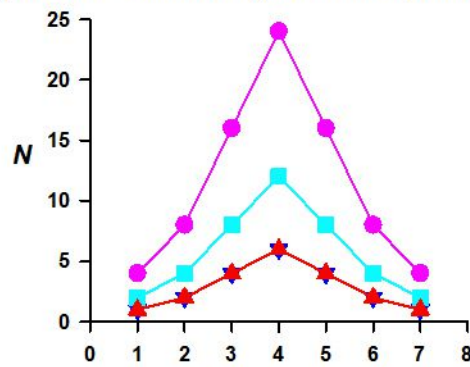
A second version of the model was also run in which the fitness penalty in the first two time periods was quartered and that in the last two time periods was halved (by adjusting the factor 480 to 1920 in the 1st and 2nd periods, and to 960 in the 6th and 7th periods, respectively). This approximates the situation in which egg-laying females are rare at the start of the season and uncommon at the end of it.

Models were run by allowing selection to alter the distribution of flowering times in successive generations until an evolutionarily stable strategy (ESS) was reached in each size category (i.e. the fitness of all plants in a size category was the same whenever they flowered in the season). This occurred when the total number of

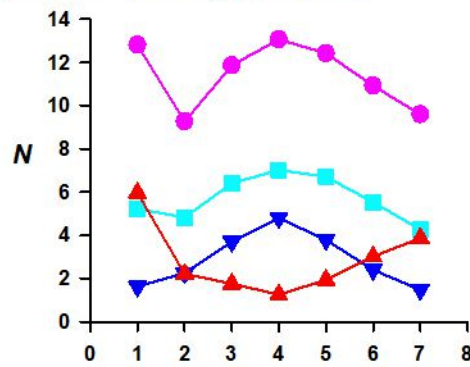
flowers on all plants in the population was the same in each time period ($T_f(t) = \text{constant}$) when the fitness penalty is constant through the season, or when $T_f(t)$ in the outer time periods (in which selection is relaxed) was quadruple/double that in the inner time periods when the penalty varied through the season. The effect of selection on the size of the plants (as measured by number of reproductive units, RU) flowering in each time period was assessed by calculating their average size on the assumption that each small, medium and large sized plant had 6, 16 and 28 RU respectively. This is double the number of flowers apparently appearing on the plants, since as flowering is not simultaneous one RU can flower in place of another as the season progresses.

The ESS solutions capture many of the traits exhibited by the *C. pratensis* populations in the study area. For the model in which the fitness penalty is constant through the season, the total number of plants (all size categories combined) flowering in each time period is nearly constant (Fig. A10.1b). Large plants flower early and late in the season and small and medium sized ones in mid season; hence, the average size of the plants is larger at the beginning and end of the season (Fig. A10.2). This approximates the pattern exhibited by large *C. pratensis* (with the exception of the return of large plants in late season), consistent with their flowering after the appearance of the butterfly (so there is no justification for reducing the fitness penalty in early season). For the model in which the fitness penalty is quartered in early season and halved in late season, the overall flowering curve (all size categories combined) is strongly bi-modal, with a sharp primary peak in early season and a diffuse secondary one in late season (Fig. A10.1c). Large plants flower primarily in early season, medium sized ones in early and late season, and small ones in mid season; hence, the average size of the plants is largest in early season, strongly depressed in mid season, and average sized in late season (Fig. A10.2). This closely matches the pattern exhibited by small *C. pratensis*, consistent with their flowering both before the appearance of the butterfly and after it has begun to decline (justifying the reduction in the fitness penalty at these times).

(a) Before selection (no fitness penalty)



(b) Fitness penalty constant



(c) Fitness penalty variable

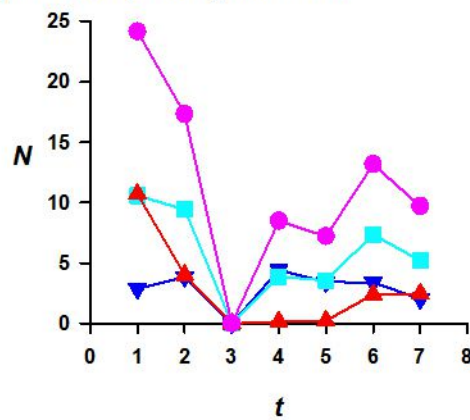


Fig. A10.1 Phenological curves for small (blue), medium (cyan) and large (red) sized *C. pratensis*, and the resultant curve for all size categories combined (pink). (a) Theoretical curves before selection (small and large curves overlapping); (b) ESS for a constant fitness penalty; (c) ESS with the fitness penalty quartered in time periods (t) 1 and 2 and halved in periods 6 and 7.

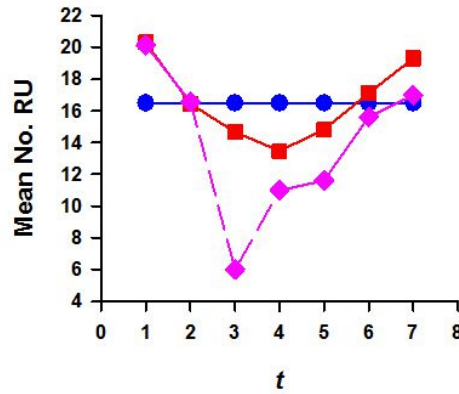


Fig. A10.2 Average size of plants in each time period (t) before selection (blue), after selection with a constant fitness penalty (red) and after selection with a variable fitness penalty (pink). No plants occur in time period 3 after selection with a variable fitness penalty (see Fig. A10.1), so this hypothetical point is joined to the others with a dashed line.

It should be noted that these model outcomes result from a 'first-come-first-served' selection process in which the largest plants are the first to occupy relatively safe early and late flowering periods due to the more intense selection pressures acting upon them. Once there, only a limited number of medium and small sized plants can join them, since as the total number of flowers (T_f) increases so does the risk of egg-laying. Had the simulation begun with a different phenological distribution to the one shown in Fig. A10.1a, an alternative result would have been obtained. For example, had the small plants initially flowered in early/late season, they would have remained in these time periods during the selection process and hampered the invasion of the large plants. However, the assumption that in the absence of selection the flowering curves of plants in all size categories would be similar and symmetrical seems reasonable.

The ESS distributions are obtained when the number of flowers in different time periods reach the specified values discussed above, which are not observed in nature. However, the simulations show that the ESS solutions are rapidly approached when the flower densities are close to their observed values. This could mean that the evolutionary process is still ongoing in nature, or that it has been throttled due to the imposition of counterbalancing selection pressures not represented in the model.

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